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(54) Title: OLFACTORY RECEPTOR SEQUENCES

(57) Abstract: The present invention provides polynucleotide sequences which encode polypeptides involved in olfactory sensation. The present invention also provides the polypeptides encoded by these polynucleotide sequences, vectors comprising these polynucleotide sequences and host cells transfected with these polynucleotide sequences. The present invention further provides for functional variants and homologues of these polynucleotide sequences and the polypeptides encoded by these polynucleotides. Libraries of polypeptides are also provided. Also included in the present invention is the use of these polypeptides and libraries of polypeptides in screening odorant molecules to determine the correspondence (scent representation, scent fingerprint or scent profile) between individual odorant receptors (the polypeptides) and particular odorant molecules. Also encompassed by the present invention is the use of the scent representation, scent fingerprint or scent profile to re-create and edit scents.

OLFACTORY RECEPTOR SEQUENCES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority benefit of United States Provisional Patent Application Serial No. 60/158,615, filed on October 8, 1999, and United States Provisional Patent Application Serial No. 60/184,809, filed on February 24, 2000. The contents of those applications are hereby incorporated by reference herein in their entirety.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

Not applicable.

TECHNICAL FIELD

The present invention is in the field of human olfactory receptors and their use in screening for olfactory agonists and antagonists. The present invention pertains to isolated nucleotide sequences which encode human olfactory receptors and also to the proteins encoded by said nucleotide sequences. The present invention also encompasses vectors comprising the nucleotide sequences of the invention and further, host cells transfected with said vectors. The present invention also allows for the determination of primary scents and the identification of the odor receptors which are encoded to detect these primary scents as well as the determination of secondary scents and the identification of combinations of odor receptors which are encoded to detect such secondary scents.

BACKGROUND ART

Our sense of smell plays an important role not only in our appreciation of our surroundings such as the smell of flowers or new mown grass, but also evolved as a survival skill. Numerous odorant molecules can be detected at extremely low concentrations, providing early warning of danger, such as the smell of smoke or contaminated food. Indeed, a potent example of this is that most pregnant women experience a heightened sense of smell, presumably to protect the fetus from the deleterious effects of food poisoning.

It is estimated that humans can detect millions of different molecular species; however, our nose can discriminate only a fraction of these different chemicals (Mombaerts *Curr. Opin. Genet. Dev.* 1999 **9**, 315-320), usually estimated at about 10,000 odorants (Axel, *Scientific American* 1995, **October**, 154-159). Odorants for terrestrial species such as humans, are volatile (air born) ligands which are detected by the olfactory system. Odorants have vastly different chemical structures and subtle differences can lead to pronounced changes in the perceived odor (Mombaerts, *supra*). For instance, when the hydroxyl group of octanol is replaced by a carboxyl group to give octanoic acid, its perceived odor changes from orange and rose-like to rancid and sweaty (Malnic *et al.*, *Cell* 1999 **96**, 713-723). The basis for these feats of sensory perception are just beginning to be understood at a cellular and molecular level.

The olfactory system contains millions of olfactory sensory neurons (OSNs) located in the olfactory epithelium of the nasal cavity. In humans, the olfactory epithelium occupies an area of approximately 5 cm^2. The OSNs are bipolar with one end extending through the supporting cell into the mucosal layer, terminating in hairlike cilia. These cilia are the site of the olfactory receptors (OR) where the odorant ligands are thought to bind (Mombaerts *Curr. Opin. Genet. Dev.* 1999 **9**, 315-320, Hildebrand *et al.*, *Annu. Rev. Neurosci.*, 1997, **20**, 595-631). The OSNs also have a single unbranched axon which leads to the olfactory bulb, a part of the brain containing approximately 2000 glomeruli where the axons terminate and initial processing of the sensory code takes place. OSNs expressing the same OR are randomly interspersed throughout the olfactory epithelium, but in both the nose and the bulb, information derived from different ORs is strictly segregated; each OSN in the nose and each glomerulus in the olfactory bulb appear to be dedicated to input from one or few OR type(s) (Malnic *et al.*, *Cell* 1999 **96**, 713-723). It also appears that the location of the glomeruli are conserved across individuals of a species, providing the first spatial processing of particular odorant patterns (Mombaerts *Curr. Opin. Genet. Dev.* 1999 **9**, 315-320). The domains in the olfactory bulb for

different odors may overlap, but the overall patterns are distinct (Hildebrand *et al.*, *supra*), therefore, it should be possible to identify and reproduce the characteristic pattern of a given odorant. Output neurons project from the olfactory bulb to the primary olfactory cortex and from there to the higher cortical areas of the brain and to the limbic system (Malnic *et al.*, *supra*; Hildebrand *et al.*, *supra*, **20**, 595-631).

Until the identification of a large family of genes encoding putative odorant receptors (Buck & Axel *Cell* 1991 **65**, 175-187), progress towards understanding the process of odor recognition was negligible. In recent years there has been an explosion in this field as more and more putative odor receptors are isolated and cloned. The odorant receptor gene products have thus far been characterized through homology as seven transmembrane domain G protein-coupled receptors (GPCR). It is estimated that there are probably 500-750 OR-like sequences in humans, while there are 500-1000 OR genes in rat and mouse (Mombaerts *Curr. Opin. Genet. Dev.* 1999 **9**, 315-320). In mice, OR-like sequences make up approximately 1% of their genome, the largest known family in the mammalian genome, surpassing the complexity of even the immunoglobin and T-cell antigen receptor gene families (Mombaerts, *supra*). The OR are concentrated on the surface of the OSN's mucus coated cilia and it is thought that odorant molecules bind to the OR in the olfactory epithelium and thereby initiate signal transduction. Current interpretation of recent experimental evidence favors the idea that each neuron expresses only one, or very few, ORs. Since mammals can detect at least 10,000 odors and there are approximately 1,000 or fewer ORs, each of the ORs must respond to several odorant molecules, and each odorant molecule must bind to several receptors. It is believed that various receptors respond to discrete parts of an odorant molecule's structure and that an odorant consists of several chemical groups each of which bind a characteristic receptor (Axel *Scientific American* 1995, **October**, 154-159; Malnic *et al.*, *Cell* 1999 **96**, 713-723).

The main signal transduction pathway mediated by OR homologues in vertebrate species involves G protein-mediated stimulation of adenylyl cyclase activity, resulting in cAMP elevation that opens cyclic-nucleotide gated channels with a non-specific cation selectivity (Mombaerts *Curr. Opin. Genet. Dev.* 1999 **9**, 315-320). However, there are still numerous unanswered questions and recently it has come to light that 38-76% of the human gene OR sequences that are being reported may be pseudogenes and therefore incapable of expressing the proteins that encode the olfactory receptors. Some of the incidences may be due to the method of extracting the genomic DNA libraries (Mombaerts, *supra*). Few pseudogenes have been found in other vertebrates and their incidence in libraries from testicular DNA is also

rare (Hildebrand *et al.*, *Annu. Rev. Neurosci.*, 1997, **20**, 595-631). cDNA should not contain pseudogenes. There are a number of examples of ORs which have been successfully expressed and reactions to certain odorant ligands have been determined (Malnic *et al.*, *Cell* 1999 **96**, 713-723; Mombaerts, *supra*; Zhao *et al.*, *Science* 1998 **279**, 237-242).

Some attempts to express the ORs in heterologous cell lines resulted in the formation of inclusion bodies rather than the insertion of the proteins into the membrane (Kiefer *et al.*, *infra*). However, purification of the receptors after expression in *E. coli* and their insertion into lipid vesicles facilitates the use of these receptors in odorant ligand screening using a combination of photoaffinity labeling and Trp fluorescence (Kiefer *et al.*, *Biochemistry* 1996 **35**, 16077-16084). In addition, a functional human OR receptor protein has been expressed in HEK-293 cells and oocytes and found to interact with odorant ligands (Wetzel et al., *J. Neurosci.* 1999 **19**, 7426-7433). There have also been, a number of successful efforts of expressing cDNA in insect Sf9 cells using *baculovirus* vectors (Mombaerts *Annu. Rev. Neuorsci.* 1999) as well as assays with neuronal tissue (Malnic *et al.*, *Cell* 1999 **96**, 713-723; Zhao et al., 1998; Firestein *et al.*, WO 98/50081). In addition, recent work accomplished the expression of chimeric mouse olfactory receptor sequences in HEK-293 cells and showed their reactivity towards a panel of odorant ligands, some at micromolar concentrations (Krautwurst *et al.*, *Cell* 1998 **95** 917-926). The drawback to expression in heterologous cell systems is the lack of working signal transduction pathways which can be used to detect responses to odorant ligands; these drawbacks can be overcome with methods known in the art (e. g. U.S. Pat. No. 5,798, 275). There are also methods of expressing and assaying functional neuronal receptors in neuronal cells, including methods for detecting particular odorant ligand specificity (Malnic *et al.*, *supra*; Zhao, *supra*; Firestein *et al, supra*).

Other publications of interest are: Chemical Senses 6: 343-349 (1981); Proc. Natl. Acad. Sci. USA 79: 670-674 (1982); Proc. Natl. Acad. Sci. USA 81(6): 1859-1863 (1984); Nature 316: 255-258 (1985); Brain Research 368: 329-338 (1986); J. Biol. Chem. 261: 1299-1305 (1986); Proc. Natl. Acad. Sci. USA 83(13): 4947-4951 (1986); J. Neurosci. 6: 2146-2154 (1986); J. Neurochem. 47: 1527-1533 (1986); Chemical Senses 13: 191-204 (1988); Biochem. J. 260:121-126 (1989); J. Biol Chem. 264: 6780-6785 (1989); Biochim. Biophys. Acta 1013: 68-72 (1989); J. Biol. Chem. 264: 18803-18807 (1989); Biochemistry 29: 7433-7440 (1990); FEBS lett. 270: 24-29 (1990); Chemical Senses 15: 529-536 (1990); Eur. J. Biochem. 196: 51-58 (1991); Nature 349: 790-793 (1991); Neurosci. Lett. 141: 115-

118 (1992); Developmental Brain Res. 73: 7-16 (1993); Proc. Natl. Acad. Sci., USA 90: 3715-3719 (1993); Human Mol. Genetics 3: 229-235 (1994); Eur. J. Biochem. 225: 1157-1168 (1994); European Journal of Biochemistry 238: 28-37 (1996); Receptors and Channels 4: 141-147 (1996); Genomics 37(2): 147-160 (1996); Protein Science 8: 969-977 (1999); Genomics 53: 56-68 (1998); Genomics 61:24-36 (1999); Genomics 63: 227-245 (2000); Trends in Neurosci. 7:35-36 (1984); Ann. Rev. Neurosci. 9:329-355 (1986); Trends Biochem. Sci. 12:63-66 (1987); Nature 351: 275-276 (1991); Nature 353: 799-800 (1991); Current Biol. 3(10): 668-674 (1993); Nature 372:321-322 (1994); Essays in Biochemistry. 33: 93-104 (1998); and Nature, 398 (6725): 285-287 (1999).

However, despite the forgoing, there has been relatively little work with human olfactory receptors, in particular in determining the sequences of large numbers of receptors, and less progress in determining the correspondence between particular human olfactory receptors and the scent(s) to which they respond.

All publications cited herein are hereby incorporated by reference in their entirety.

DISCLOSURE OF THE INVENTION

An object of the invention is to determine the correspondence between ORs and the scent(s) to which they respond. Once this is accomplished, scents can be both analyzed and re-created for enhancing human experiences or eliciting particular responses. The present invention pertains to isolated polynucleotide sequences encoding polypeptides involved in olfactory sensation. The present invention also pertains to the proteins encoded by said nucleotide sequences. The present invention also encompasses vectors comprising the nucleotide sequences of the invention and further, host cells transfected with said vectors. The present invention also allows for the determination of primary scents and the identification of the odor receptors which are encoded to detect these primary scents as well as the determination of receptor complex scent components and the identification of combinations of odor receptors which are encoded to detect such receptor complex scent components scents.

The invention provides isolated polynucleotide sequences encoding polypeptides involved in olfactory sensation that are isolated from human olfactory epithelial tissue. The invention further provides expression vectors containing such nucleotide sequences. Also provided by the invention are purified polypeptides encoded by the nucleotide sequences. The invention further provides transformed cells which comprise a suitable host cell transfected with a suitable expression vector containing the nucleotide sequence encoding the receptor. The present invention also encompasses nucleotide sequences isolated from human olfactory epithelial tissue which encode receptors capable of binding odorant molecules. The invention further provides expression vectors containing such nucleotide sequences and homologues of both the polynucleotides and polypeptides. Further, the invention provides a means of using the nucleotide sequences of the invention in a method of screening odorant ligands to determine the specific binding of odorant molecules to a particular receptors, and further, determining the component odorant molecules of subjectively experienced smells, determining the combination odorant molecules and receptor stimulation or inhibition to re-create a particular scent. The binding of odorant molecules by the receptors encompassed in the present invention includes binding resulting in both the agonism (excitation/activation) and antagonism (inhibition/blocking) of receptor function(s) upon binding of the molecule.

Accordingly, the invention includes an isolated polynucleotide comprising a sequence encoding a polypeptide which is involved in olfactory sensation. The OR polypeptides encoded are found within the sequences depicted in polynucleotide sequences SEQ ID NO:1 through SEQ ID NO: 73 and SEQ ID NO:111 through SEQ ID NO:152, or a nucleotide sequence at least 95% homologous to said sequences. The invention also encompasses the translation products of those sequences. The invention further comprises expression vectors comprising said sequences, host cells containing such expression vectors and/or expressing the polypeptide encoded therein, or phage displaying the polypeptide encoded by the sequences. The use of functional fragments of receptors is also encompassed by the invention. Preparations of receptors, further including biological or synthetic molecules which maintain the stability and functional structure of the receptors, are also included in the invention. The invention further encompasses fragments of said polynucleotides which can be used as probes or primers to identify additional polynucleotide sequences through techniques known in the art, including those fragments depicted in SEQ ID NOs: 74-105.

The invention also includes additional isolated polynucleotide comprising a sequence encoding a polypeptide which is involved in olfactory sensation. The OR polypeptides

encoded are found within the sequences depicted in polynucleotide sequences SEQ ID NO:153 through SEQ ID NO: 1084, or a nucleotide sequence at least 95% homologous to said sequences. The invention also comprises the translation products of those sequences. The invention further comprises expression vectors comprising said sequences, host cells containing such expression vectors and/or expressing the polypeptide encoded therein, or phage displaying the polypeptide encoded by the sequences. The use of functional fragments of receptors is also encompassed by the invention. Preparations of receptors, further including biological or synthetic molecules which maintain the stability and functional structure of the receptors, are also included in the invention.

The invention also encompasses an isolated and purified olfactory receptor polypeptide scomprising the sequence of SEQ ID NO: 1085 through SEQ ID NO: 2008, or a polypeptide sequence that is at least about 95% homologous to a polypeptide sequence of the group consisting of SEQ ID NO: 1085 through SEQ ID NO: 2008 and having olfactory receptor function. Host cells expressing such polypeptides and phages displaying such polypeptides are also encompassed by the invention. The use of functional fragments of receptors is also encompassed by the invention. Preparations of receptors, further including biological or synthetic molecules which maintain the stability and functional structure of the receptors, are also included in the invention.

Scents can be captured, analyzed and recorded by a sensory device using various methods. Scent capture can be initiated by the user or by an automatic sensing system. A scent can be analyzed in terms of its interaction with olfactory neurons of a mammalian, preferably human, olfactory system, or by the expression of individual receptors under appropriate conditions and appropriate assay conditions in multiwell plates or in terms of its perception by a panel of mammalian, preferably human, subjects. The interaction with olfactory neurons can be determined experimentally, in vitro, by determining the interaction of an odorant with olfactory receptors of a given type. Alternatively, the interaction with olfactory receptor can be determined using a computer simulation which provides information regarding the interaction of an odorant with the olfactory receptors. A panel of subjects can be used to represent odors in terms of their perception. The data so generated can be used to represent a scent in a manner which can be recorded in digital or other format, stored in media such as computer memory, disks, or printed format, and transmitted over a data network. The representation of the scent can be used to re-create the scent at a local or remote site using an emitter module. The

representation of the scent allows for scent editing, where desirable aspects of an odor are enhanced or added and undesirable aspects are attenuated or eliminated.

Accordingly, the invention also embraces libraries of olfactory receptors suitable for determining the interaction pattern of a composition with the receptors, comprising the expression products of at least two polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084, where the polynucleotides encode functional olfactory receptors; or functional fragments of the expression products. Libraries of at least 50, 100, 200, or 500 receptors are also encompassed by the invention.

Also encompassed by the invention are libraries of olfactory receptors suitable for determining the interaction pattern of a composition with the receptors, comprising at least two polypeptides of SEQ ID NO: 1085 through SEQ ID NO: 2008, where the polypeptides are functional olfactory receptors; or functional fragments of the polypeptides. Libraries of at least 50, 100, 200, or 500 receptors are also encompassed by the invention.

The invention also embraces methods for determining the binding pattern of a composition with olfactory receptors, involving exposing the composition to an olfactory receptor library, and determining whether the composition binds to each olfactory receptor, thereby determining the overall binding patter of the composition. In additional embodiments, the method also involves determining the approximate binding constant with which the composition, or the various chemicals within the composition, bind to the receptors; determining whether a receptor or functional fragment thereof is activated; and determining the absolute amount of activation, or amount of activation relative to another receptor or a control substance. The composition can consist essentially of one compound or chemical, or can comprise at least two compounds or chemicals.

The invention also embraces DNA arrays or DNA chips comprising the DNA segments derived from any combination of, or each of, SEQ ID NO: 153 through SEQ ID NO: 1084. The invention also embraces a method of determining differences among one or more individuals with respect to their olfactory faculties, comprising the steps of comparing the olfactory DNA of each individual against the array or chip.

The invention also embraces a method to determine single nucleotide polymorphisms in olfactory receptors, comprising the steps of uniquely amplifying olfactory receptor sequences from DNA obtained from one or more individuals, based on

primers designed according to the first 25 bases and the last 25 bases of any combination of, or each of, SEQ ID NO: 153 through SEQ ID NO: 1084, and determining the similarities and differences between said amplified DNA and the corresponding receptor from SEQ ID NO: 153 through SEQ ID NO: 1084.

Brief Description of the Drawings

Figure 1 depicts the isolated polynucleotide sequences, which encode polypeptides involved in olfactory sensation, corresponding to SEQ ID NOs: 1 - 73.

Figure 2 depicts the isolated polynucleotide sequences, which encode polypeptides involved in olfactory sensation, corresponding to SEQ ID NOs: 111 - 152.

Detailed Description of the Invention

The present invention provides isolated polynucleotides comprising sequences that encode polypeptides which are involved in olfactory sensation and which can be used to screen odorant ligands, *e.g.*, odorant receptor agonists and antagonists.

Definitions

The term "olfactory receptor" (OR) refers to a polypeptide involved in olfactory sensation. An "olfactory receptor polynucleotide" or "OR polynucleotide" is a polynucleotide encoding a polypeptide involved in olfactory sensation.

The term "odorant ligand" as employed herein refers to a molecule that has the potential to bind to an olfactory receptor. Equivalent terms employed herein include "odorant", "odorant molecule" and "odorant compound". The term "binding" or "interaction" as used herein with respect to odorant ligands refers to the interaction of ligands with the receptor polypeptide where the ligands may serve as either agonists and/or antagonists of a given receptor or receptor function. An odorant ligand may thus directly cause a perception of odor (an agonist), or may block the perception of odor (an antagonist). An odorant ligand may include, but is not limited to, molecules which interact with polypeptides involved in olfactory

sensation. Odorant ligands and molecules which interact with olfactory receptors are generally small, approximately 1000 Daltons, more preferably approximately 750 Daltons, more preferably approximately 500 Daltons, or even more preferably approximately 300 Daltons, hydrophobic molecules with a variety of functional groups. Small changes in structure can induce profound changes in odorant ligand binding and hence in the odor perceived by an individual.

A more detailed description of these sequences, as well as how these sequences were obtained, is provided below.

As used herein, a "polynucleotide" is a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. The terms "polynucleotide", "nucleotide" and "nucleic acid" as used herein are used interchangeably. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term "polynucleotide" includes double- , single-stranded, and triple-helical molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double stranded form. Not all linkages in a polynucleotide need be identical.

The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, primers, and adaptors. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The use of uracil as a substitute for thymine in a deoxyribonucleic acid is also considered an analogous form of pyrimidine.

In the context of polynucleotides, a "linear sequence" or a "sequence" is an order of nucleotides in a polynucleotide in a 5' to 3' direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polynucleotide. A "partial sequence" is a linear sequence of part of a polynucleotide which is known to comprise additional residues in one or both directions.

If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by

conjugation with a labeling component. Other types of modifications included in this definition are, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, cabamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., α-anomeric nucleic acids, peptide nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s).

Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH groups can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups.

Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, but not limited to, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carboxcyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside.

Although conventional sugars and bases will be used in applying the method of the invention, substitution of analogous forms of sugars, purines and pyrimidines can be advantageous in designing a final product, as can alternative backbone structures like a polyamide backbone such as those used in peptide nucleic acids (PNAs).

A polynucleotide or polynucleotide region has a certain percentage (for example, 75%, 80%, 85%, 90%, 95% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases are the same in comparing the two sequences.

Homology, as described herein, means that the polypeptide sequences that are encoded by the nucleic acids demonstrate a certain relatedness (i.e., there exists regions of conserved amino acids), but not the same amino acid identity. There is complete or 100% homology at a particular amino acid residue when the amino acids of sequences being compared are the same (there is identity) or represent a conservative amino acid substitution (there is homology). A

"conservative amino acid substitution" occurs when a particular amino acid is substituted by an alternate amino acid of similar charge density, hydrophobicity/hydrophilicity, size and/or configuration (e.g., Val for Ile). A "nonconservative amino acid substitution" occurs when a particular amino acid is substituted by an alternative amino acid of differing properties, that is, charge density, hydrophobicity/hydrophilicity, size and/or configuration (e.g., Val for Tyr). The nucleic acid sequences within the scope of the present invention include those nucleic acids which differ in exact sequence from those listed in SEQ ID NO:1 through SEQ ID NO:73 and SEQ ID NO:111 through SEQ ID NO:152 but which encode identical or homologous polypeptide amino acid sequences.

A "primer" is a short polynucleotide, generally with a free 3' -OH group, that binds to a target potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target.

An "adaptor" is a short, partially-duplexed polynucleotide that has a blunt, double-stranded end and a protruding, single-stranded end. It can be ligated, through its double-stranded end, to the double-stranded end of another polynucleotide. This provides known sequences at the ends of thus modified polynucleotides. Often adaptors contain specific sequences for primer binding and/or restriction endonuclease digestion.

A "probe" when used in the context of polynucleotide manipulation refers to a polynucleotide which is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and enzymes.

"Transformation" or "transfection" refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, lipofection, transduction, infection or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host cell genome.

A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide, a homologous polypeptide or a fragment thereof. For purposes of this invention, and to avoid cumbersome referrals to complementary strands, the anti-sense (or complementary) strand of such a polynucleotide is also said to encode the

sequence; that is, a polynucleotide sequence that "encodes" a polypeptide includes both the conventional coding strand and the complementary sequence (or strand).

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, it may be interrupted by non-amino acids, and it may be assembled into a complex of more than one polypeptide chain. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an N-terminal to C-terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A "partial sequence" is a linear sequence of part of a polypeptide which is known to comprise additional residues in one or both directions.

"Recombinant," as applied to a polynucleotide or gene, means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature.

A "vector" is a self-replicating nucleic acid molecule that can be used to transfer an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of a nucleic acid molecule into a cell, vectors that function primarily for the amplification of nucleic acid, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors that provide more than one of the above functions.

"Expression vectors" are defined as polynucleotides which, when introduced into an appropriate host cell, can be transcribed into a mRNA capable of being translated into a polypeptide(s). An expression vector also comprises control elements operatively linked to the coding region to enable and/or facilitate expression of the polypeptide in the target cell. These can include transcriptional, translational, posttranscriptional, and posttranlational control elements, as are known in the art. An "expression system" usually connotes a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

A "host cell" includes an individual cell or cell culture which can be or has been a recipient for vector(s) or for incorporation of nucleic acid molecules and/or proteins. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

A "cell line" or "cell culture" denotes eukaryotic cells, derived from higher, multicellular organisms, grown or maintained in vitro. It is understood that the descendants of a cell may not be completely identical (either morphologically, genotypically, or phenotypically) to the parent cell. Cells described as "uncultured" are obtained directly from a living organism, and are generally maintained for a limited amount of time away from the organism (i.e., not long enough or under conditions for the cells to undergo substantial replication).

As used herein, "expression" includes transcription and/or translation.

"Heterologous" means derived from (i.e., obtained from) a genotypically distinct entity from the rest of the entity to which it is being compared. For example, a polynucleotide may be placed by genetic engineering techniques into a plasmid or vector derived from a different source, thus becoming a heterologous polynucleotide. A promoter which is linked to a coding sequence with which it is not naturally linked is a heterologous promoter.

An "isolated" or "purified" polynucleotide, polypeptide or cell is one that is substantially free of the materials with which it is associated in nature. By substantially free is meant at least 50%, preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 99%, and even more preferably at least 99.9% free of the materials with which it is associated in nature. As used herein, an "isolated" polynucleotide or polypeptide also refers to recombinant polynucleotides or polypeptides, which, by virtue of origin or manipulation: (1) are not associated with all or a portion of a polynucleotide or polypeptide with which they are associated in nature, (2) are linked to a polynucleotide or polypeptide other than that to which they are linked in nature, or (3) do not occur in nature, or (4) in the case of polypeptides, arise from expression of recombinant polynucleotides. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, by specific activity or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this invention are increasingly more preferred.

Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by processes such as chemical synthesis or recombinant expression.

A "reagent" polynucleotide, polypeptide, or antibody, is a substance provided for a reaction, the substance having some known and desirable function in the reaction. A reaction mixture may also contain a "target", such as a polynucleotide, antibody, polypeptide, or assembly of polypeptides that the reagent is capable of reacting with. For example, in some types of diagnostic tests, the presence and/or amount of the target in a sample is determined by adding a reagent, allowing the reagent and target to react, and measuring the amount of reaction product (if any).

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of an amplification reaction such as PCR, or the enzymatic cleavage of a polynucleotide by a ribozyme.

When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" to another polynucleotide if hybridization can occur between one of the strands of the first polynucleotide and the second. The degree to which one polynucleotide is complementary with another is quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules of A-T, A-U and G-C.

A "stable duplex" of polynucleotides, or a "stable complex" formed between any two or more components in a biochemical reaction, refers to a duplex or complex that is sufficiently long-lasting to persist between formation of the duplex or complex and subsequent detection, including any optional washing steps or other manipulation that may take place in the interim.

A substance is said to be "selective" or "specific" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An odorant ligand "specifically

binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances.

As used herein, "naturally occurring," "native," or "wild type" refers to endogenous polynucleotides and the protein(s) expressed thereby. These terms include full-length and processed polynucleotides and polypeptides. Processing can occur in one or more steps, and these terms encompass all stages of processing. For instance, polypeptides having or lacking a signal sequence are encompassed by the invention. "Non-naturally occurring", "non-native", or "non-wild type" refer to all other polynucleotides and polypeptides.

A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using one or more primers, and a catalyst of polymerization, such as a reverse transcriptase or a DNA polymerase, and particularly a thermally stable polymerase enzyme. Methods for PCR are taught in U.S. Patent Nos. 4,683,195 (Mullis) and 4,683,202 (Mullis et al.). All processes of producing replicate copies of the same polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "amplification."

According to this invention, a "genomic DNA library" is a clone library which contains representative nucleotide sequences from the DNA of a given genome. It is constructed using various techniques that are well known in the art, for instance, by enzymatically or mechanically fragmenting the DNA from an organism, organ, or tissue of interest, linking the fragments to a suitable vector, and introducing the vector into appropriate cells so as to establish the genomic library. A genomic library contains both transcribed DNA fragments as well as nontranscribed DNA fragments.

In comparison, a "cDNA library" is a clone library that differs from a genomic library in that it contains only transcribed DNA sequences and no nontranscribed DNA sequences. It is established using techniques that are well known in the art, i.e., selection of mRNA (e.g. by polyA) making single stranded DNA from a population of cytoplasmic mRNA molecules using the enzyme RNA-dependent DNA polymerase (i.e., reverse transcriptase), converting the single-stranded DNA into double-stranded DNA, cloning the resultant molecules into a vector, and introducing the vector into appropriate cells so as to establish the cDNA library. Alternately, a cDNA library need not be cloned into a vector and/or established in cells, but can be screened using PCR with gene-specific primers, as is well known in the art.

An "individual" is a vertebrate, preferably a mammal, more preferably a human.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, such as: "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M.J. Gait, ed., 1984); "Animal Cell Culture" (R.I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Gene Transfer Vectors for Mammalian Cells" (J.M. Miller & M.P. Calos, eds., 1987); "Current Protocols in Molecular Biology" (F.M. Ausubel et al., eds., 1987 and annual updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994); "Current Protocols in Immunology" (J.E. Coligan et al., eds., 1991).

Basis for identification and description of the polynucleotides and polypeptides

The polynucleotide sequences were identified using oligonucleotide primers which were complementary to OR membrane-spanning regions. A number of different primers were used to elicit a variety of nucleotide sequences which encode polypeptides involved in olfactory sensation. The identification and isolation of nucleotide sequences which encode polypeptides involved in olfactory sensation and the polypeptides that they encode is vital for determining the response of receptors to odorant molecules, the elucidation of scent representations, profiles, or fingerprints, the reproduction of scent representations, profiles, or fingerprints and the editing of scent representations, profiles, or fingerprints.

Polynucleotides encoding polypeptides involved in olfactory sensation

The present invention provides isolated polynucleotides encoding polypeptides which are involved in olfactory sensation, vectors containing these polynucleotides, host cells containing these polynucleotides, and compositions comprising these polynucleotides. These polynucleotides are isolated and/or produced by chemical and/or recombinant methods, or a combination of these methods. The present invention includes polynucleotides isolated from the human olfactory epithelium which encode polypeptides which are involved in olfactory sensation, vectors containing these polynucleotides, host cells containing these polynucleotides, and compositions comprising these polynucleotides. Unless specifically stated otherwise,

"polynucleotides" shall include all embodiments of the polynucleotides of this invention. These polynucleotides are useful as probes, primers, in expression systems, and, in a preferred embodiment, in screening methods as described herein. In one embodiment the polynucleotides of the present invention can be isolated by creating a cDNA library using template RNA from human olfactory epithelium tissue. A detailed example is related in Example 1, below.

The advantage of constructing a cDNA library for isolation of the desired nucleotide sequences is that the likelihood of obtaining pseudogenes is greatly reduced compared to using a genomic DNA library for the same purpose. cDNA libraries contain only mRNA expressed in the tissue used for the construction of the library, in this case, the human olfactory epithelium. The preferred olfactory epithelium tissue should express only those nucleotide sequences which are relevant for olfactory function, thereby excluding nonfunctioning pseudogenes and also GPCRs which may be similar in primary structure (amino acid sequence) but are not encoded in OSNs. As the number of GPCRs utilized in human signal transduction pathways is extremely wide and varied, cDNA libraries constructed using olfactory tissue are preferable for isolating nucleotide sequences that encode polypeptides which are involved in olfactory sensation, inasmuch as genomic libraries can contain abundant nucleotide sequences which encode for a variety of GPCRs performing numerous functions, and are likely to contain pseudogenes.

The isolation of polynucleotide sequences which encode polypeptides involved in olfactory sensation is described in Example 1. Accordingly, this invention provides isolated polynucleotides that contain sequences encoding polypeptides or portions thereof which are involved in olfactory sensation, wherein the polypeptide is at least 10 amino acids in length, and wherein the polynucleotide sequences are depicted in SEQ ID NOs:1-73 and SEQ ID NOs:111-152.

The invention includes modifications to said polynucleotides described above such as deletions, substitutions, additions, or changes in the nature of any nucleic acid moieties. A "modification" is any difference in nucleotide sequence as compared to a polynucleotide shown herein to encode a polypeptide involved in olfactory sensation, and/or any difference in the nucleic acid moieties of the polynucleotide(s), wherein such a modified polynucleotide encodes a polypeptide involved in olfactory sensation or a variant of said polypeptide that is useful in the practice of the invention. Such changes can be useful to facilitate cloning and modify expression of polynucleotides encoding polypeptides which are involved in olfactory

sensation. Such changes also can be useful for conferring desirable properties to the polynucleotide(s), such as stability. The definition of polynucleotide provided herein gives examples of these modifications. Hence, the invention also includes variants of the nucleic acid sequences disclosed herein, which include nucleic acid substitutions, additions, and/or deletions.

The invention also encompasses polynucleotides encoding polypeptides involved in olfactory sensation, including polynucleotides that are full-length, processed, coding, non-coding (including flanking region) or portions thereof, provided that these polynucleotides contain a region encoding at least a portion of a polypeptide involved in olfactory sensation. (That is, the region encodes a functional fragment of an olfactory receptor or other polypeptide involved in olfactory sensation.) Also embodied are the mRNA, cDNA and genomic DNA sequences and fragments thereof that include a polynucleotide sequence comprising a coding sequence for a portion of a polypeptide involved in olfactory sensation.

Genes encoding human olfactory receptors, and optionally including related genomic sequences such as regulatory sequences, can be obtained using olfactory receptor cDNAs as hybridization probes. Under high stringency hybridization conditions, an OR cDNA will hybridize to its cognate OR gene. Use of lower stringency hybridization conditions allows the isolation of OR genes that are related to, but not identical with, the gene corresponding to a particular OR cDNA.

Conditions for hybridization are well-known to those of skill in the art and can be varied within relatively wide limits. Hybridization stringency refers to the degree to which hybridization conditions disfavor the formation of hybrids containing mismatched nucleotides, thereby promoting the formation of perfectly matched hybrids or hybrids containing fewer mismatches; with higher stringency correlated with a lower tolerance for mismatched hybrids. Factors that affect the stringency of hybridization include, but are not limited to, temperature, pH, ionic strength, and concentration of organic solvents such as formamide and dimethylsulfoxide. As is well known to those of skill in the art, hybridization stringency is increased by higher temperatures and/or lower ionic strengths. See, for example, Ausubel et al., supra; Sambrook et al., supra; M.A. Innis et al. (eds.) PCR Protocols, Academic Press, San Diego, 1990; B.D. Hames et al. (eds.) Nucleic Acid Hybridisation: A Practical Approach, IRL Press, Oxford, 1985; and van Ness et al., (1991) Nucleic Acids Res. 19:5143-5151. The degree of stringency can be adjusted not only during a hybridization reaction, but also in post-hybridization washes, as is known to those of skill in the art.

The invention also encompasses polynucleotides encoding polypeptides involved in olfactory sensation, functionally equivalent variants and derivatives of full-length polypeptides involved in olfactory sensation and functionally equivalent fragments. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, non-deleterious non-conservative substitutions, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs, will not significantly affect properties of the encoded polypeptide. Polypeptides homologous to the polypeptides encoded by the polynucleotides described herein can also be identified using algorithms and methods well-known to those of skill in the art, such as those described in Ausubel, "Current Protocols in Molecular Biology," Chapter 19; see also Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. (1993) "Identification of protein coding regions by database similarity search." Nature Genet. 3:266-272; Madden, T.L., Tatusov, R.L. & Zhang, J. (1996) "Applications of network BLAST server" Meth. Enzymol. 266:131-141; Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402; and Zhang, J. & Madden, T.L. (1997) "PowerBLAST: A new network BLAST application for interactive or automated sequence analysis and annotation." Genome Res. 7:649-656. A preferred method of determining homology is the BLAST set of similarity search programs (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410. Polypeptides which are 40% homologous, 50% homologous, 60% homologous, 70% homologous, 80% homologous, 90% homologous, 95% homologous, or 99% homologous to the polypeptides encoded by the polynucleotides described herein are encompassed by the invention.

Nucleotide substitutions that do not alter the amino acid residues encoded can be useful for optimizing gene expression in different systems. Suitable substitutions are known to those of skill in the art and are made, for instance, to reflect preferred codon usage in the particular expression systems. In another example, alternatively spliced polynucleotides can give rise to different functionally equivalent fragments or variants of an polypeptide involved in olfactory sensation. Alternatively processed polynucleotide sequence variants are defined as polynucleotide sequences corresponding to mRNAs that differ in sequence from one another but are derived from the same genomic region, for example, mRNAs that result from: 1) the

use of alternative promoters; 2) the use of alternative polyadenylation sites; and/or 3) the use of alternative splice sites.

Preparation of polynucleotides involved in olfactory sensation

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR.

Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing polynucleotides which encode polypeptides involved in olfactory sensation using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating, particle bombardment, liposome mediation, or electroporation. Once introduced, an exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide encoding a polypeptide involved in olfactory sensation can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al. (1989).

Alternatively, PCR allows amplification of DNA sequences. PCR technology is well known in the art and is described in U.S. Pat. Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as *PCR: The Polymerase Chain Reaction*, Mullis et al. eds., Birkhausw Press, Boston (1994).

RNA can be obtained in a number of ways in an appropriate vector and the vector is transformed into a suitable host cell. When the inserted DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., (1989), for example. RNA can also be obtained through in vitro reactions. For example, the polynucleotide, which encodes a polypeptide involved in olfactory sensation, can be inserted into a vector that contains appropriate transcription promoter sequences. Commercially available RNA polymerases will specifically initiate transcription at their promoter sites and continue the transcription process through the adjoining DNA polynucleotides. Placing the polynucleotide sequences which encode polypeptides involved in

olfactory sensation between two such promoters allows the generation of sense or antisense strands of desired RNA.

Cloning and expression vectors comprising polynucleotide sequences encoding polypeptides involved in olfactory sensation

The present invention further includes a variety of vectors containing polynucleotides encoding polypeptides involved in olfactory sensation. These vectors can be used for expression of recombinant polypeptides as well as a source of polynucleotides which encode polypeptides involved in olfactory sensation. Cloning vectors can be used to obtain replicate copies of the polynucleotides, which encode polypeptides involved in olfactory sensation, they contain, or as a means of storing the polynucleotides in a depository for future recovery. Expression vectors (and host cells containing these expression vectors) can be used to obtain polypeptides produced from the polynucleotides they contain. Suitable cloning and expression vectors include any known in the art, e.g., those for use in in vitro, bacterial, mammalian, yeast and insect expression systems. Specific vectors and suitable host cells are known in the art and need not be described in detail herein. For example, see Gacesa and Ramji, *Vectors*, John Wiley & Sons (1994).

Cloning and expression vectors typically contain a selectable marker (for example, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector), although such a marker gene can be carried on another polynucleotide sequence co-introduced into the host cell. Only those host cells into which a selectable marker has been introduced will survive and/or grow under selective conditions. Typical selectable markers encode protein(s) that (a) confer resistance to antibiotics or other toxins substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper marker gene will depend on the host cell, and appropriate genes for different hosts are known in the art. Cloning and expression vectors also typically contain a replication system recognized by the host.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate in an appropriate host, may possess a single target for one or more particular restriction endonucleases, and/or may carry genes for a marker

that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, m13mp18, m13mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

Expression vectors generally are replicatable polynucleotide constructs that contain a polynucleotide encoding an polypeptide involved in olfactory sensation of interest. The polynucleotide, which encodes a polypeptide involved in olfactory sensation, encoding the polypeptide is operatively linked to suitable transcriptional controlling elements, such as promoters, enhancers and terminators. For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons. These controlling elements (transcriptional and translational) may be derived from the gene encoding polypeptides involved in olfactory sensation, or they may be heterologous (i.e., derived from other genes and/or other organisms). A polynucleotide sequence encoding a signal peptide can also be included to allow a polypeptide involved in olfactory sensation to cross and/or lodge in cell membranes or be secreted from the cell. A number of expression vectors suitable for expression in eukaryotic cells including yeast, insect, avian, plant and mammalian cells are known in the art. Common vectors, such as YEp13 and the Sikorski series pRS303-306, 313-316, 423-426 can also be used. Vectors pDBV52 and pDBV53 are suitable for expression. Another example of an expression vector/host cell system is the baculovirus (*e.g.*, nuclear polyhedrosis virus)/insect cell (*e.g.*, sf9 cells) system.

Human olfactory receptor polypeptides are expressed from olfactory receptor cDNA by methods well-known to those of skill in the art. A cDNA or portion thereof is inserted in an expression vector using standard molecular cloning techniques. Coupled in vitro transcription and translation of such a vector results in expression of the OR protein encoded by the cDNA. In vivo expression of a OR polypeptide is accomplished by inserting an OR cDNA into a eucaryotic or procaryotic expression vector, of which many are known in the art, to genereate an OR expression construct. The OR expression construct is introduced into an appropriate host cell in which the OR sequences are expressed (by transcription and translation) and optionally secreted, and the expressed OR polypeptide is obtained from the cell growth medium and/or from cell lysates.

A number of expression vectors are known in the art. Prokaryotic expression vectors include, but are not limited to, T7 RNA polymerase/T7 promoter-based vectors, bacteriophage λ-based vectors and various types of fusion vectors. Fusion vectors include, but are not limited to, lacZ and trpE fusion vectors, maltose binding protein fusion vectors, glutathione-S-transferase fusion vectors, and thioredoxin fusion vectors. Baculovirus-based vectors are used for expression in insect cell systems. Expression in mammalian cells (such as HEK, COS and CHO cells) utilizes vectors containing a mammalian origin of replication (such as, for example, a SV40 origin), an efficient promoter (optionally including one or more enhancer sequences), mRNA processing signals (e.g., splice sites and polyadenylation sites), one or more selectable markers, and optionally a prokaryotic replicon to allow propagation and manipulation of the construct in prokaryotic cells. Alternatively, expression in mammalian cells is achieved through the use of any of a number of mammalian viral vectors including, but not limited to, retroviruses, lentiviruses, Semliki Forest viruses, vaccinia viruses, adenoviruses and adeno-associated viruses.

Vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, direct injection, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent, such as a virus). The choice of means of introducing vectors or polynucleotides encoding polypeptides involved in olfactory sensation will often depend on the host cell, as will be well known to those of skill in the art.

Host cells transformed with polynucleotides encoding polypeptides involved in olfactory sensation

Another embodiment of this invention are host cells transformed with (i.e., comprising) polynucleotides encoding polypeptides involved in olfactory sensation, and/or vectors having polynucleotide(s) sequences encoding polypeptides involved in olfactory sensation, as described above. Both prokaryotic and eukaryotic host cells may be used. Prokaryotic hosts include bacterial cells, for example *E. coli*, *B. subtilis*, and mycobacteria. Among eukaryotic hosts are yeast, insect, avian, plant and mammalian cells. Host systems are known in the art and need not be described in detail herein.

The host cells of this invention can be used, *inter alia*, as repositories of polynucleotides encoding polypeptides involved in olfactory sensation, and/or vehicles for

production of polynucleotides encoding polypeptides involved in olfactory sensation, and/or polypeptides involved in olfactory sensation . They may also be used as vehicles for *in vivo* delivery of polypeptides involved in olfactory sensation .

Uses for and methods using polynucleotides encoding polypeptides involved in olfactory sensation

To determine whether a vector containing polynucleotides is capable of expressing in eukaryotic cells, cells such as, for example, COS-7 (primate origin), CHO (rodent origin), HEK-293 (human origin), or HeLa (human origin) cells can be transfected with the vector. Expression of a polypeptide(s) encoded by the vector is then determined by, for example, RIA, ELISA, immunofluorescence of fixed cells, or western blotting of cell lysate using an antibody as a probe. Antibodies can be obtained using, as immunogen, peptide sequences synthesized from the protein sequences encoded by the known polynucleotide sequence. Polypeptides can be purified by, for example, phase partitioning, affinity methods, gel filtration and ion exchange, as well as additional methods known by those skilled in the art. Further characterization of the expressed polypeptide can be achieved by purification of the polypeptide using techniques known in the art.

Polypeptides involved in olfactory sensation

The present invention encompasses polypeptides involved in olfactory sensation. Expression of said polypeptides is localized in the olfactory neurons located in the olfactory epithelium, as described earlier. The polypeptides may comprise any novel sequence encoded by a nucleotide sequence as depicted in SEQ ID NO:1 through SEQ ID NO:73 and SEQ ID NO:111 through SEQ ID NO:152.

The invention includes modifications to polypeptides involved in olfactory sensation including functionally equivalent fragments of the polypeptides involved in olfactory sensation which do not significantly affect their properties and variants which may have enhanced or decreased activity. Collectively, these modifications may be termed "analogs" of or a fragment of polypeptides involved in olfactory sensation. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for

one another include but are not limited to: glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine; lysine/arginine; and phenylalanine/tyrosine. Such conservative substitutions are known in the art, and preferably, the amino acid substitutions would be such that the substituted amino acid would possess similar chemical properties as that of the original amino acid. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modified polypeptides involved in olfactory sensation are made using established procedures in the art.

The invention also encompasses fusion proteins comprising one or more polypeptides involved in olfactory sensation. For purposes of this invention, an fusion protein contains one or more polypeptides involved in olfactory sensation and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Useful heterologous sequences include, but are not limited to, sequences that provide for secretion from a host cell, intracellular trafficking, and stability/degradation. Other useful heterologous sequences are ones which facilitate purification. Examples of such sequences are known in the art and include those encoding epitopes such as Myc, HA (derived from influenza virus hemagglutinin), His-6, or FLAG. Other heterologous sequences that facilitate purification are derived from proteins such as glutathione S-transferase (GST), maltose-binding protein (MBP), or the Fc portion of immunoglobulin.

Preparation of polypeptides involved in olfactory sensation

The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by recombinant methods (i.e., single or fusion polypeptides) or by chemical synthesis. Polypeptides, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, a polypeptide can be produced by an automated polypeptide synthesizer employing the solid phase method. Polypeptides can also be made by chemical synthesis using techniques known in the art.

Polypeptides can also be made by expression systems, using recombinant methods. The availability of polynucleotides encoding polypeptides permits the construction of expression vectors encoding intact (i.e., native) polypeptide, functional equivalents and functional fragments thereof, modified forms or recombinant forms. A polynucleotide encoding the desired polypeptide, or a fusion protein, can be ligated into an expression vector suitable for any convenient host. Both eukaryotic and prokaryotic host systems can be used. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification or isolation of the polypeptides expressed in host systems can be accomplished by any method known in the art (e.g. partitioning exclusion, ion exchange chromatograph, gel filtration, etc.). Other controlling transcription or translation segments, such as signal sequences that direct the polypeptide to a specific cell compartment (i.e., for secretion), can also be used. Examples of prokaryotic host cells are known in the art and include, for example, *E. coli* and *B. subtilis*. Examples of eukaryotic host cells are known in the art and include yeast, avian, insect, plant, and animal cells such as COS7, HeLa, CHO, HEK-293 and other mammalian cells.

Alternatively, in vitro expression systems may also be used to produce polypeptides involved in olfactory sensation. A plasmid containing a polynucleotide encoding polypeptides involved in olfactory sensation, under the control of an appropriate promoter, can be transcribed and the resultant RNA translated in vitro through the use of commercially available reagents. Such methods can be used to produce relatively pure samples of the polypeptide and are known in the art.

Preferably, the polypeptides are at least partially purified from other cellular constituents. In one embodiment, the polypeptides are at least 70%, more preferably at least 80%, even more preferably at least 90% or most preferably at least 95% pure. In this context, purity can be calculated as a weight percent of the total protein content of the preparation. More highly purified polypeptides may also be obtained and are encompassed by the present invention. Methods of protein purification are known in the art and are not described in detail herein. For membrane-bound proteins, the lipid content of the preparation, which is required to maintain the structure and function of the protein, is excluded from the purity calculation. That is, if a preparation weighing 10 mg has 5 mg lipid, 4 mg of desired protein, and 1 mg of undesired proteins, the purity is calculated as 80% (desired protein content divided by total protein content). Preparations of biological or synthetic molecules suitable for maintaining structure and function of membrane proteins are described in Etemadi AH (1985) *Adv Lipid*

Res 1985;21:281-428; Villalobo A (1990) *Biochimica Et Biophysica Acta*, 1017(1):1-48; Montal M (1987) *Journal Of Membrane Biology* 98(2): 101-115; Scotto AW et al. (1987) *Biochemistry* 26(3): 833-839; Jain MK and Zakim D (1987) *Biochimica Et Biophysica Acta* 906(1): 33-68; Czerski L and Sanders CR (2000) *Anal Biochem* 284(2):327-33 (lipid-detergent mixtures or "bicelles"); Hrafnsdottir S and Menon AK (2000) *J Bacteriol* 182(15):4198-206 (proteoliposomes); Puu G et al. (2000) *Biosens Bioelectron* 15(1-2):31-41 (protein-lipid preparations on solid surfaces); Schafmeister CE et al. (1993) *Science* 262(5134):734-8 ("peptitergents").

Uses of polypeptides involved in olfactory sensation

The polypeptides of this invention have a variety of uses. They can be used, for example, to screen odorant ligands in order to determine the scent representations, scent profiles or scent fingerprints of particular odorant molecules and further to characterize the effect of functional groups and chemical characteristics on perceived smell. Methods for screening odorant compounds using odorant receptors in neuronal cells are known in the art (Firestein et al., WO 98/50081; Duchamp-Viret *et al.*, *Science* 1999, **284** 2171-2174; Sato *et al.*, *J. Neurophys.* 1994 **72** 2980-2989; Malnic *et al*, *Cell* 1999 **96** 713-723; Zhao *et al.*, *Science* 1998 **279**, 237-242). There are also methods which can be employed to screen odorant compounds which do not require neuronal cells and are known in the art (Kauvar et al., U. S. Pat. No. 5,798,275; Kiefer *et al.*, *Biochemistry* 1996 **35** 16077-16084; Krautwurst *et al.*, *Cell* 1998 **95** 917-926),

Analysis of the scent can be performed in a number of ways. Various embodiments of the scent analysis system are presented. Examples of how these embodiments might operate are also presented, although it should be emphasized that the invention is not limited by any particular theory of olfactory perception or scent analysis.

Olfactory Space

The sensory subsystem comprises a series of olfactory receptors, which selectively bind with the chemical component(s) making up the scent. The scent can be characterized in terms of which of the approximately 1,000 olfactory receptors the scent component(s) bind to, and the strength of the interaction of the component(s) with those receptors. Each olfactory receptor can be considered an orthogonal basis vector; the entire set of olfactory receptors can be considered a set of basis vectors spanning "olfactory space." This is analogous to vectors

pointing along the x, y, and z directions in three-dimensional space, where any point in space can be represented by a combination of the x, y, and z basis vectors (with each of the x, y, and z vectors multiplied by the appropriate scalar quantity). The intensity of interaction of a scent with an olfactory receptor determines the magnitude of the vector along that particular "axis" in olfactory space. Thus, every scent can be uniquely described by a vector representation in olfactory space.

A representation of a scent in such a manner that the scent can later be re-created is defined as scent profiling. The aforementioned vector representation is one example of a scent profile.

Primary Scents

For the purposes of this invention, a receptor primary scent component is defined as a chemical that interacts with one and only one scent receptor. A receptor complex scent component is defined as a chemical that interacts with more than one scent receptor; the receptor complex scent component can interact with each of the scent receptors to different degrees, to equal degrees, or can interact with some receptors to the same degree and others to different degrees.

Olfactory receptors are proteins which fall in the class of seven transmembrane domain G protein-coupled receptors, and are found in olfactory neurons *in vivo*. Binding of an odorant to an olfactory receptor causes second messenger systems to become activated or inhibited in the cell, leading to increased cellular production of second messenger molecules such as cyclic AMP. These second messenger systems in turn lead to the depolarization of the olfactory neuron, or other changes in the state of the neuron, which provides the signal to the nervous system that the odorant has been detected.

With a complete set of receptor primary scent components, any scent can be re-created with the knowledge to the degree to which it interacts with each olfactory receptor. The instant invention encompasses such complete sets of receptor primary scent components. Other embodiments of the invention encompass sets of receptor primary scent component chemicals which provide the ability to re-create a particularly desired subset of scents, but not necessarily all possible scents. Still more embodiments encompass sets of receptor primary scent component chemicals which provide the ability to approximate particular scents, while not necessarily exactly re-creating the interaction profile of the particular scents.

In some cases, a receptor complex scent will be an acceptable approximation to a receptor primary scent. That is, if a given receptor complex scent interacts with a first scent receptor strongly, but interacts with other scent receptors less strongly, it can be considered an approximation to a receptor primary scent component for the first receptor. Such a receptor complex scent component is described by the term receptor quasi-primary scent component. One embodiment of the invention encompasses sets of receptor quasi-primary scent component chemicals suitable for re-creating all scents. Another embodiment of the invention encompasses sets of receptor quasi-primary scent component chemicals suitable for re-creating a particularly desired subset of scents, but not necessarily all possible scents. Yet another embodiment encompasses sets of receptor quasi-primary scent component chemicals which provide the ability to approximate particular scents, while not necessarily exactly re-creating the interaction profile of the particular scents.

The identification of receptor primary or quasi-primary scent component chemicals provides the most conceptually straightforward method of re-creating scents. However, another embodiment of the invention encompasses the use of receptor complex scent components for re-creating scents. An example of such an embodiment would be re-creation of a scent that activates olfactory receptors designated OR1, OR2, OR3, OR4, OR5 and OR6 (for the sake of illustration, it is assumed that the olfactory receptors are stimulated to an equal extent). If one is in possession of two receptor complex scent component chemicals (RCSC's) where RCSC1 activates OR1 and OR5, and RCSC2 activates OR2, OR3, OR4, and OR6, then one can reproduce the original scent by mixing RCSC1 and RCSC2 to re-create the original olfactory receptor activation profile. In practice, the profiles of various receptor complex scent components will be much more complicated than the forgoing example, and components which inhibit olfactory activation as well as stimulate activation can be included in the sets. However, once receptor activation profiles of sufficient receptor complex scent components are known, computer algorithms can be utilized to create the appropriate combination of receptor complex scent components. Using vector representations of the olfactory receptor activation profiles for a set of receptor complex scent components, one can create linear combinations of such receptor complex scent components in order to represent a particular scent. For the example given above, such a vector representation would look like (1, 0, 0, 0, 1, 0) for the first receptor complex scent component and (0, 1, 1, 1, 0, 1) for the second receptor

complex scent component, while the vector representation of the scent to be re-created is (1, 1, 1, 1, 1, 1). If x_1 and x_2 are the relative proportions of the first receptor complex scent component and the second receptor complex scent component, respectively, to be combined to re-create the scent, then the problem can be represented as a series of linear equations:

$$\begin{aligned} 1x_1 + 0x_2 &= 1 \\ 0x_1 + 1x_2 &= 1 \\ 0x_1 + 1x_2 &= 1 \\ 0x_1 + 1x_2 &= 1 \\ 1x_1 + 0x_2 &= 1 \\ 0x_1 + 1x_2 &= 1 \end{aligned}$$

and the solutions for x_1 and x_2 are $x_1 = 1$, $x_2 = 1$. Solutions to systems of linear equations have been thoroughly studied and many algorithms are available for implementation on computers, including algorithms which evaluate the accuracy of an approximate solution when an exact solution cannot be determined. (See, e.g., Dettman, J.W., *Introduction to Linear Algebra and Differential Equations*, Dover Pubs., 1986; Press W.H. et al., *Numerical Recipes in C: The Art of Scientific Computing*, 2nd ed., Cambridge University Press, 1993; Vetterling (ed.) *Numerical Recipes in C: The Art of Scientific Computing/Disk V 2.02*, Cambridge University Press, 1997.) These methods can also be used to determine whether a set of receptor complex scent components is suitable for re-creating a given scent. For example, if the scent to be recreated is represented by the vector (1, 1, 1, 1, 1, 2), there will be no solution to the resulting system of linear equations using the two receptor complex scent components in the illustration above. In this instance, one or more additional receptor scent components will need to be identified in order to be able to re-create the scent in terms of the receptor primary scent components. Alternatively, the scent represented by (1, 1, 1, 1, 1, 1) may be an acceptable approximation to the scent represented by (1, 1, 1, 1, 1, 2). Integers are used in this example for clarity, but the vectors can contain any real number representing a measured intensity; for example, (1.1, 0.997, 1.08, 1.2, 0.88888..., 2.00001) may be an acceptable approximation to the scent represented by (1, 1, 1, 1, 1, 2).

It will be readily appreciated that the choice of a complete set of receptor primary, quasi-primary, or complex scent component chemicals (capable of generating all scents) versus a partial set of receptor primary, quasi-primary, or complex scent component chemicals (capable of generating, exactly or approximately, a subset of scents) depends on the application for which scent re-creation is desired.

A special category of receptor scent components are chemicals which bind to a receptor without activating it. If these non-activating chemicals prevent chemicals which do activate the receptors from binding, the non-activating chemicals act to "turn off" those receptors. These non-activating chemicals, or receptor binding antagonists, are particularly useful in editing scents, as they can be added to a scent to attenuate or eliminate particular aspects of the scent. In the vector example above, if a particular receptor antagonist blocks OR2, OR3, and OR4, but not OR1, OR5 or OR6, then it can be represented in vector format as (0, -1, -1, -1, 0, 0). In the reproduction of (1, 1, 1, 1, 1, 2) from the vectors (1, 0, 0, 0, 1, 0) and (0, 1, 1, 1, 0, 1), the following combination can be used: 1 x (1, 0, 0, 0, 1, 0) + 2 x (0, 1, 1, 1, 0, 1) + 1 x (0, -1, -1, -1, 0, 0) to yield the vector (1, 1, 1, 1, 1, 2). In some instances, enough of a particular receptor binding antagonist is used to eliminate any possibility of activation by a receptor scent component, in which case the vector entry for the receptor(s) which are blocked by that antagonist contains 0 in the vector position corresponding to that receptor(s).

Perceptive primary scents are defined as scents that give a single scent perception, for example, the scent "lemon" as perceived by a human. A perceptive primary scent can be composed of one or more receptor primary scent components, one or more receptor complex scent components, or a mixture of one or more receptor primary scent components and one or more receptor complex scent components. Since perceptive primary scents are to some extent subjective, identification of perceptive primary scents can be performed by using a panel of subjects who evaluate and describe scents. A perceptive complex scent is made up of more than one perceptive primary scent. The boundaries between a perceptive primary scent and a perceptive complex scent are also to some extent subjective; for example, one person may describe a scent as "pizza," while another person may describe the same scent as "sausage, cheese and tomato sauce." That is, one person may perceive a scent as a perceptive primary scent for "pizza," while another person may perceive the same scent as a perceptive complex scent made up of several individual perceptive primary scents. In order to standardize perceptive scents, a panel of five or more, preferably ten or more, more preferably fifty or

more, still more preferably one hundred or more, people can be surveyed to label various perceptive scents. When a plurality, preferably a majority, more preferably 66 2/3 % or greater, still more preferably 95 % or greater, even more preferably 99% or greater, of the panel identifies a scent as the same scent (e.g., of a panel of 100 people, 95 describe a scent as "pizza," while the other 5 describe the scent otherwise), the scent can be labeled as a perceptive scent (the perceptive scent can be primary or complex, depending on whether the panel identifies it as a single scent or a mixture of scents).

In fields where existing classification schemes already exist, the perceptive primary and complex scents can be indexed according to those schemes. For example, the SFP (Société Française des Parfumeurs) has drawn up a classification system based on 5 main groups, sub-divided into classes. Such a classification can be used for selecting perceptive primary scents and used as guides for combining the scents.

Selecting Chemicals for Scent Re-creation

A scent which has been represented as a set of basis vectors in olfactory space can in principle be re-created simply by mixing the receptor primary scent components, receptor quasi-primary scent components, or receptor complex scent components needed to interact the olfactory receptors in the same pattern as the original scent. Such an approach requires 1) a method to generate a representation of the original scent in olfactory space, and 2) suitable receptor primary scent component chemicals which can be mixed in the appropriate manner.

Identification of receptor scent components can be performed by various methods. One such method assays the interaction of candidate components with each olfactory receptor. The receptors can be expressed *in vitro* and assays can be set up to monitor the interaction of various candidate components with each individual receptor. Chemicals which interact with one and only one olfactory receptor are receptor primary scent components, while chemicals which interact with more than one olfactory receptor are receptor complex scent components (and can possibly be receptor quasi-primary scent components, depending on the interaction profile it displays with the olfactory receptors). Such an approach can use methods known in the art, for example those of Breer *et al,* Ann. N. Y. Acad. Sci. (1998) 855:175-81 or Malnic *et al., Cell* (1999) 96(5):713-23. Breer *et al.* expressed olfactory receptors in Sf9 cells and evaluated the second-messenger response to various odorants. Malnic *et al.* isolated olfactory neurons from mice and utilized calcium imaging to study the response of the neurons to different odorants, while using RT-PCR to determine which olfactory receptor was expressed

in the neuron under study. U.S. Patent No. 5,798,275 describes a method for evaluating interaction of compounds with members of a reference panel of proteins. WO 98/50081 discloses methods for detecting particular odorant ligand specificity for particular odorant receptors in nasal epithelium tissue of mammals such as rats and mice.

Selection of Receptor Primary Scents by in silico Methods

An alternative method utilizes *in silico* screening techniques--that is, computer simulation methods--for selecting candidate components. Protein-ligand screening can be used to select compounds which bind to particular receptors in order to identify receptor primary scent components. Examples of such programs are DOCK, AutoDock, GOLD, FlexX, LUDI, GROWMOL, and HOOK. (See Wang, J., Kollman, P.A., Kuntz I.D., "Flexible ligand docking: a multistep strategy approach," *Proteins* 36(1):1-19 (1999) and references therein.) These programs function by taking a protein structure and either matching compounds of known structure to the protein structure to determine the protein-ligand interaction, or by "growing" a molecule in the active site or binding site of a protein to determine what molecule will best interact with the protein.

Olfactory receptor proteins are membrane proteins, and experimental determination of the three-dimensional structures of membrane proteins has lagged the corresponding structural determination of water-soluble proteins for various reasons. However, alternative methods for constructing the three-dimensional structures of proteins are available. The primary (amino acid) sequences of many olfactory receptors are known. This information can be used to model a three-dimensional structure of a receptor protein using various algorithms and computer programs known in the art. The resulting model structure can then be used as the basis for evaluating interaction of candidate components with the receptor.

Alternatively, given known chemical structures which give rise to a particular odor, analysis of the structures can indicate the particular portion of the chemical structure which is responsible for the odor. This is analogous to "pharmacore analysis" used in medicinal chemistry to determine the important portion of drugs.

Methods for developing compounds which bind to receptors and other proteins of known structure, and determining interactions between ligands and receptors, are described in various references The DOCK program evaluates the fit of a ligand into a protein molecule of known structure (see Gschwend, D.A., Good, A.C. and Kuntz, I.D., "Molecular Docking Towards Drug Discovery", *J. Mol. Recognition* 9, 175-86 (1996); Kuntz, I.D., Meng, E.C., and

B.K. Shoichet, "Structure-Based Strategies For Drug Design and Discovery", *Acc. Chem. Res.* 27, 117-123 (1994); and Kuntz, I.D., "Structure-based strategies for drug design and discovery", *Science* 257, 1078-1082 (1992); see also http://www.cmpharm.ucsf.edu/kuntz/dock.html). Using a known (or modeled) structure of an olfactory receptor, DOCK can be used to screen for compounds which bind to the receptor. The program AMBER (see Cornell, WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW and Kollman PA. "A second generation force field for the simulation of proteins and nucleic acids," *Journal of the American Chemical Society* 117, 5179-5197 (1995); Computer Simulation of Biomolecular Systems, A. Wilkinson, P. Weiner, W. Van Gunsteren, eds. Volume 3, p. 83-96, P. Kollman, R. Dixon, W. Cornell, T. Fox, C. Chipot and A. Pohorille; Bayly CI, Cieplak P, Cornell WD and Kollman PA. "A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges - the RESP model," *Journal of Physical Chemistry* 97(40), 10269-10280 (1993); Cornell WD, Cieplak P, Bayly CI and Kollman PA. "Application of RESP charges to calculate conformational energies, hydrogen bond energies, and free energies of solvation," *Journal of the American Chemical Society* 115(21), 9620-9631 (1993); see also http://www.amber.ucsf.edu/amber/amber.html) can be used to calculate more precise interaction energies between candidate ligands. Other examples of such methods are described in, for example, U.S. Patent No. 5,866,343, directed to determining the energetically favorable binding site between two molecules; U.S. Patent No. 5,854,992, a system and method for structure-based drug design which takes into account binding free energy as it "grows" candidate molecules into a receptor binding site; and U.S. Patent No. 5,495,423, which describes a method for ligand design (principally applicable to peptidic ligands).

The foregoing methods typically depend on a known three-dimensional structure for the receptor. When such a structure cannot or has not been determined experimentally, a structure can be modeled using computer algorithms. Blundell TL, Sibanda BL, Sternberg MJ, Thornton JM, "Knowledge-based prediction of protein structures and the design of novel molecules," *Nature* 326(6111):347-52 (1987); Shortle D, "Structure prediction: The state of the art," *Curr Biol* 9(6):R205-9 (1999), Morea V, Leplae R, Tramontano A, "Protein structure prediction and design," *Biotechnol Annu Rev* 4:177-214 (1998) and Onuchic JN, Luthey-Schulten Z, Wolynes PG, "Theory of protein folding: the energy landscape perspective," *Annu Rev Phys Chem* 48:545-600 (1997) address various methods of predicting protein structure from sequence data.

Various implementations for predicting protein structure from amino acid sequences are discussed in U.S. Patent Nos. 5,878,373 and 5,884,230.

If the structure, or even the identity, of the targeted receptor cannot be determined, alternative computational techniques can be used to generate information regarding possible ligands which will interact with the receptor. Quantitative structure-activity relationships (QSAR; see Green, S.M. and Marshall, G.R., "3-D QSAR: A current perspective," Trends Pharmacol Sci 16:285 (1995); and 3D QSAR in Drug Design: Theory, Methods and Applications, Kubinyi, H. Ed.; Escom, Leiden.), including QSAR refinements such as comparative molecular field analysis (ComFA) (Cramer, R. D. et al. "Comparative Molecular Field Analysis ComFA 1. Effect Of Shape On Binding Of Steroids To Carrier Proteins," *J. Am. Chem. Soc.* 110: 5959 (1988)); and pharmacophore mapping (Martin YC, Bures MG, Danaher EA, DeLazzer J, Lico I, Pavlik PA, "A fast new approach to pharmacophore mapping and its application to dopaminergic and benzodiazepine agonists," *J Comput Aided Mol Des* 7(1):83-102 (1993)) have been used to design pharmacophores that can interact with the receptor. U.S. Patent No. 5,699,268 provides a method for producing computer-simulated receptors which functionally mimic biological receptors; the simulated receptors are essentially abstractions of structurally useful information from compounds which are known to interact with a receptor. U.S. Patent No. 5,901,069 describes a method of automatically refining a set of chemicals using structure/activity data. U.S. Patent No. 5,862,514 describes a method of simulating synthesis of compounds of desired biological activity and evaluating their activity via further simulations.

Application of structure-function relationships to classification of odors has been described by Chastrette M., Rallet E. "Structure-minty odour relationships: Suggestion of an interaction pattern," *Flavour and Fragrance Journal*, 13(1):5-18 (1998); Chastrette M., De Saint Laumer J.Y.,; Peyraud J.F., "Adapting the structure of a neural network to extract chemical information. Application to structure-odour relationships," *SAR QSAR Environ Res* 1 (2-3):221-231 (1993), Chastrette M., "Trends in structure-odor relationships," *SAR QSAR Environ Res* 6(3-4):215-254 (1997) and Jain et al., "A shape-based machine learning tool for drug design," *J Comput Aided Mol Des* 8(6):635-652 (1994). These methods can be useful in determining the "chemical distance" between odors. For example, isoamyl acetate is typically experienced as a banana-like odor, while octyl acetate is typically experienced as an orange-like odor, which gives a measure of how the chain length of the alkoxy portion of the ester influences perception.

Olfactory Receptors and Libraries of Olfactory Receptors

The olfactory receptors of the invention can be used to analyze and describe the interaction of scent odorant molecules with each receptor. This can be done individually, receptor-by-receptor and odorant molecule by odorant molecule. However, a combinatorial approach provides a much more powerful method of analyzing and describing the interaction of scent odorant molecules with olfactory receptors.

In one embodiment, the invention comprises libraries of olfactory receptors. These libraries are used to screen compositions for interaction with receptors. A composition can be a single compound (essentially a pure chemical), or a mixture of two or more compounds or chemicals. The compositions can be presented to the library in vapor form, or in solutions, typically aqueous solutions.

The method for determining the binding pattern of a composition with olfactory receptors comprises the steps of: exposing the composition to an olfactory receptor library; and determining whether the composition binds to each olfactory receptor of the library, thereby determining the overall binding patter of the composition. While it is desirable to determine whether the composition binds to each of the olfactory receptors, in certain cases, determining the binding pattern to a subset of the receptors is suitable. Such a situation can arise if the complete pattern is not needed, or if the experiment cannot determine binding to a receptor for a particular reason. (Determining the binding to a subset is equivalent to reducing the olfactory receptor library to that subset of receptors.)

Typically, the libraries are prepared as arrays, where the position of each olfactory receptor is known on the array. The arrays can take the form of multiwell plates, solid substrates such as chips or wafers, or any other form allowing identification of the receptor location. The arrays can be prepared in order to simply assess binding, or can be prepared in order to assess degree of activation as described above, using, for example, the technique of Malnic *et al.*, *Cell* 1999 **96**, 713-723. Alternatively, an *in silico* array of structures can be prepared, using the known primary structure of the receptors and the modeling techniques described above.

The libraries contain at least two olfactory receptors. In increasing order of preference, the libraries contain at least 5, 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1500, 1600, 1800, or 2000 olfactory receptors. The

receptors are presented as ordered arrays. For example, a 96-well plate can contain 96 receptor preparations. Upon exposure to a composition, the plate can be scanned, and the response of each receptor in each well can be evaluated. This leads to a 96-element vector description of the composition in terms of those 96 olfactory receptors.

In one embodiment, binding to the olfactory receptors is assessed. In another embodiment, the approximate binding constant of the composition to the olfactory receptors is determined. In yet another embodiment, the degree of activation of the olfactory receptor by the composition is determined. For receptor antagonists, binding will occur, but no activation will occur; the invention embraces the identification of such antagonists.

The compositions for use are varied. A set of all volatile compounds can be used. A standard set of perfumes or odorants can be used. A set of commercially used scents can be used. Sets of compounds particularly useful in the invention are disclosed in co-pending United States Patent Application Serial No. 09/620,753. However, it must be emphasized that the invention is not limited to any one set or classification of compounds.

Preferred subsets of olfactory receptor polynucleotide sequences include:

SEQ ID NOS: 163, 331, 414, 425,672, 762, 919, and 1027;

SEQ ID NOS: 809 and 1067;

SEQ ID NO: 744;

SEQ ID NOS: 207, 336, 441, and 615;

SEQ ID NOS: 157, 168, 197, 221, 250, 334, 340, 412, 413, 459, 491, 618, 690, 694, 759, 760, 761, 767, 819, 860, 872, 873, 917, 936, 939, 940, 947,952, 958, 959, 1023, 1034, 1038, 1043, and 1044;

SEQ ID NOS: 783, 785, 882, 888, 922, and 925;

SEQ ID NOS: 707, 748, 752, 755, 756, 790, and 997;

SEQ ID NOS: 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, and 1084;

SEQ ID NOS: 163, 239, 331, 335, 368, 381, 385, 414, 425, 514, 572, 596, 603, 628, 638, 642, 672,674, 689, 744, 762, 809, 835, 885, 896, 919, 920, 938, 948, 972, 999, 1007, 1014, and 1027;

SEQ ID NOS: 164, 173, 176, 180, 182, 184, 185, 188, 190, 194,207, 210, 213, 214, 215, 217, 219, 220, 223, 226, 227, 229, 230, 234, 235, 240, 249, 255, 265, 270, 273, 274,

276, 277, 279, 281, 289, 291, 293, 294, 298, 302, 307, 311, 318, 319, 321, 330, 336, 339, 341, 342, 343, 348, 351, 356, 359, 361, 365, 366, 367, 368, 370, 372, 373, 374, 375, 376, 378, 379, 380, 382, 383, 384, 385, 388, 391, 392, 393, 398, 400, 401, 403, 408, 420, 423, 427, 428, 431, 434, 435, 438, 439, 440, 441, 447, 448, 450, 455, 458, 464, 465, 468, 471, 473, 474, 475, 478, 479, 481, 482, 484, 485, 492, 494, 499, 502, 508, 511, 512, 513, 515, 526, 532, 534, 541, 543, 545, 546, 550, 552, 553, 557, 558, 560, 563, 564, 568, 572, 576, 582, 583, 584, 585, 586, 588, 599, 600, 605, 606, 607, 608, 609, 610, 615, 620, 621, 631, 632, 636, 638, 640, 642, 645, 648, 650, 651, 652, 654, 656, 657, 661, 662, 664, 668, 679, 680, 686, 687, 689, 691, 696, 699, 700, 702, 706, 713, 720, 721, 723, 729, 734, 738, 745, 768, 772, 773, 775, 791, 798, 799, 823, 857, 898, 900, 901, 903, 914, 931, 933, 937, 941, 945, 948, 956, 965, 969, 983, 992, 993, 994, 999, 1003, 1005, 1009, 1010, 1011, 1019, 1028, 1035, 1037, 1052, 1061, 1062, and 1063

SEQ ID NOS: 157, 161, 163, 168, 197, 200, 205, 218, 221, 242, 250, 331, 334, 340, 412, 413, 414, 419, 425, 452, 453, 454, 456, 459, 462, 491, 591, 618, 622, 663, 665, 667, 670, 672, 690, 694, 695, 709, 759, 760, 761, 762, 767, 819, 820,822, 826, 832, 846, 847, 860, 872, 873, 877, 881, 887, 908, 911, 913, 917, 919, 921, 936, 939, 940, 942, 944, 947, 951, 952, 955, 958, 959, 960, 964, 975, 977, 979, 986, 1023, 1027, 1034, 1038, 1043, 1044, 1049, and 1051;

SEQ ID NOS: 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 332, 333, 334, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 382, 383, 384, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406,

407, 408, 409, 410, 411, 412, 413, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 597, 598, 599, 600, 601, 602, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 629, 630, 631, 632, 633, 634, 635, 636, 637, 639, 640, 641, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 673, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 939, 940, 941, 942, 943, 944, 945, 946, 947, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 1000, 1001, 1002, 1003, 1004, 1005,

1006, 1008, 1009, 1010, 1011, 1012, 1013, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, and 1064;

and any and all combinations of the foregoing sets.

The polypeptide translation products of those polynucleotide sequences form sets of preferred olfactory receptor polypeptides, as well as any and all combinations of those polypeptide sets. The preferred sets of polypeptide translation products, and any and all combinations thereof, are also preferred sets for use as libraries of olfactory receptors for scent analysis.

Scent Fingerprinting

It will be appreciated that in many instances, analysis of a scent (whether in terms of receptor primary scent components, receptor quasi-primary scent components, receptor complex scent components, or other scent representations) is of great utility in and of itself, in addition to the utility of that analysis in scent re-creation. Thus, another embodiment of the invention encompasses "scent fingerprinting," which comprises analysis of a scent profile when re-creation of that scent may not be necessary or desirable. The distinction between scent profiling, as defined above, and scent fingerprinting, as defined here, is that scent profiling is a representation of a scent relative to a mammalian olfactory system in such a manner as to provide useful information about the interaction of the scent with that olfactory system, such as sufficient information to enable re-creation of the scent from receptor primary scent components. In contrast, scent fingerprinting can, but does not necessarily, provide such information.

Various applications and examples of scent fingerprinting can include, but are not limited to, the following illustrative situations. Natural gas is widely used as a heating and fuel supply, but is in itself odorless. Utility companies routinely add small amounts of odorants such as mercaptans to allow detection of natural gas leaks in households. Should a leak occur at an unattended site, however, potentially dangerous quantities of natural gas can accumulate. In such areas, a device which can recognize odorants would be useful.

Another use of scent fingerprinting is quality control of a manufacturing process. Many food items, such as freshly-baked bread and pastries, sauces, and cheeses, have distinct

odors. A manufacturer can record a scent fingerprint for a given food item, e.g. spaghetti sauce for packaging in jars. The quality of the product can then be monitored at various stages in manufacture and storage, and deviations from the established scent fingerprint can be used to alert the manufacturer to problems in manufacture or storage. Quality control scent fingerprints are not limited to food items, but can be used in any circumstance where a volatile component of an item of manufacture can be used as a quality control indicator, e.g., perfume, deodorants, solvent mixtures, etc.

While scent fingerprints need not be meaningful in terms of a mammalian olfactory system, it will be readily appreciated that a scent profile, which does represent a scent in a manner relevant to an olfactory system, is a special type of scent fingerprint. Additionally, the response of a device which yields a scent fingerprint of an odor (such as the "artificial nose" described in U.S. Pat. Nos. 5,571,401, 5,698,089, 5,788,833, 5,891,398 and 5,911,872) can be calibrated against the response of a mammalian olfactory system in order to transform the scent fingerprint generated by the device into a true scent profile which can be utilized to re-create an odor using receptor primary scent components, receptor quasi-primary scent components, or receptor complex scent components. The invention encompasses such data transformations.

Scent Editing

Representation of a scent as a scent profile provides the capability of editing the scent. A scent profile which represents a scent in terms of perceptive primary scent components is the most straightforward representation to edit. An example is the perceptive complex primary scent of "burned pizza" comprised of perceptive primary scent components of sausage, cheese, tomato sauce, and burned dough. In order to edit the scent to provide a more pleasant re-creation, the perceptive primary scent component of burned dough would simply be eliminated.

Other scent profiles can be edited using a knowledge of the perception of a particular components. Using our six-receptor example, suppose that the (1, 0, 0, 0, 1, 0) receptor complex scent component is known to provide an unpleasant aspect of the scent, while the (0, 1, 1, 1, 0, 1) component is known to provide the pleasant aspect of the scent. The first complex scent component can be omitted from the edited scent profile, leaving (0, 1, 1, 1, 0, 1) as the edited scent profile. (This would also alter the index values for scent re-creation, from 1 and 1, to 0 and 1.) More complex editing situations can be manipulated using computer algorithms as discussed above.

Individual scent components can be omitted, added, weakened, or intensified, and different scent components can be adjusted in different manners or degrees, depending on the desired result. The editing can be done interactively, with each edited scent emitted by the emitter module for evaluation by the user, or can be done automatically, with removal/weakening or addition/intensifying of particular components specified in advance, on either an absolute scale or relative to other components.

The following examples are presented to illustrate, but not to limit, the invention.

EXAMPLES

Example 1: Isolation of human olfactory receptor cDNAs

Total RNA was extracted from human olfactory epithelium and polyA$^+$ RNA was obtained by oligo-dT selection. This RNA served as template for cDNA synthesis using reagents from the SMART cDNA Library construction kit (Clontech K1051-1; Palo Alto, CA). The Superscript II™ reverse transcriptase (Life Technologies, Gaithersburg, MD) was used for first-strand synthesis.

Double-stranded cDNA was passed through a Chroma-Spin$^+$ STE-100 column (Clontech) to remove unreacted primers and cDNA fragments shorter that 100 nucleotides. The olfactory epithelial cDNA population was then subjected to amplification using primers homologous to conserved regions in GPCRs. The first primer set was homologous to transmembrane segment 2 (TM2) and the second set was homologous to TM 7.5. The TM2 primer set contained 32 oligonucleotides, representing all possible nucleotide sequences capable of encoding the TM2 amino acid sequence motif P-M-Y-F/L-F/Y-F/L, and designed to be non-degenerate at their 3' ends. Sequences of the TM2 primers are as follows:

CCN ATG TAY TTN CTC CTA	SEQ ID NO: 74
CCN ATG TAY TTN CTC CTC	SEQ ID NO: 75
CCN ATG TAY TTN CTC CTG	SEQ ID NO: 76
CCN ATG TAY TTN CTC CTT	SEQ ID NO: 77
CCN ATG TAY TTN CTC TTA	SEQ ID NO: 78
CCN ATG TAY TTN CTC TTC	SEQ ID NO: 79
CCN ATG TAY TTN CTC TTG	SEQ ID NO: 80
CCN ATG TAY TTN CTC TTT	SEQ ID NO: 81
CCN ATG TAY TTN CTT CTA	SEQ ID NO: 82
CCN ATG TAY TTN CTT CTC	SEQ ID NO: 83
CCN ATG TAY TTN CTT CTG	SEQ ID NO: 84

CCN ATG TAY TTN CTT CTT	SEQ ID NO: 85
CCN ATG TAY TTN CTT TTA	SEQ ID NO: 86
CCN ATG TAY TTN CTT TTC	SEQ ID NO: 87
CCN ATG TAY TTN CTT TTG	SEQ ID NO: 88
CCN ATG TAY TTN CTT TTT	SEQ ID NO: 89
CCN ATG TAY TTN TTC CTA	SEQ ID NO: 90
CCN ATG TAY TTN TTC CTC	SEQ ID NO: 91
CCN ATG TAY TTN TTC CTG	SEQ ID NO: 92
CCN ATG TAY TTN TTC CTT	SEQ ID NO: 93
CCN ATG TAY TTN TTC TTA	SEQ ID NO: 94
CCN ATG TAY TTN TTC TTC	SEQ ID NO: 95
CCN ATG TAY TTN TTC TTG	SEQ ID NO: 96
CCN ATG TAY TTN TTC TTT	SEQ ID NO: 97
CCN ATG TAY TTN TTT CTA	SEQ ID NO: 98
CCN ATG TAY TTN TTT CTC	SEQ ID NO: 99
CCN ATG TAY TTN TTT CTG	SEQ ID NO: 100
CCN ATG TAY TTN TTT CTT	SEQ ID NO: 101
CCN ATG TAY TTN TTT TTA	SEQ ID NO: 102
CCN ATG TAY TTN TTT TTC	SEQ ID NO: 103
CCN ATG TAY TTN TTT TTG	SEQ ID NO: 104
CCN ATG TAY TTN TTT TTT	SEQ ID NO: 105

The TM7.5 primer set was designed to contain the reverse complement of all sequences capable of encoding the TM7.5 amino acid sequence motif P-F/L/I/V-I/V-F/Y-S/T-L. The sequences of the TM7.5 primers are as follows:

YYTNGTNYTNRYNCYGATANATNATNGGRTT	SEQ ID NO: 106
YTRTTNCKNAGNWRTANATRAANGGRTT	SEQ ID NO: 107
TCYTTRTTNCKNAGNGWRTANAYNASNGGRTT	SEQ ID NO: 108
TCNTSRTTNCKNARNSARTANATNATNGGRTT	SEQ ID NO: 109
RTTNCKNARNSWRTANATRAANGGRTT	SEQ ID NO: 110

Reagents and enzymes for amplification were from the Advantage cDNA amplification kit (Clontech). A primary amplification reaction was constructed as follows:

5 µl olfactory epithelial cDNA (10-20 µg/ml)

5 µl 10X PCR reaction buffer (Clontech)

1 µl TM2 primer set (10 µM)

1 µl TM7.5 primer set (10 µM)

1 µl dNTP mix (10 mM each dATP, dCTP, dGTP, dTTP)

36 µl PCR-grade H_2O

1 µl Advantage polymerase mix (Clontech)

Amplification was conducted in a PE 480 thermal cycler, using 28 cycles of 95°C for 15 sec, 45°C for 45 sec and 72°C for 2 min. After cycling, the amplification mixture was treated for 1 hour at 37°C with 10 Units of BspEI and 10 Units of PstI restriction enzymes, to degrade non-specific amplification products.

The primary amplification products were size-fractionated by agarose gel electrophoresis, and amplification products having a length between 600 and 800 base pairs were selected for secondary amplification.

The secondary amplification reaction was conducted identically to the primary amplification reaction, except that the size-selected primary amplification product was used as template. Secondary amplification reactions containing products which generated a specific gel band of between 600 and 800 base pairs were extracted once with phenol/chloroform and once with chloroform, and nucleic acids were precipitated from the reactions by addition of 0.1 volume of 3M NaOAc (pH 4.8), 20 μg glycogen, and 1.5 volumes of cold 95% ethanol. The precipitate was collected by centrifugation, dried and resuspended in 15 μl distilled water. After the precipitate dissolved, 3 μl loading dye was added, and the sample was subjected to electrophoresis on a 1.0% low-melting agarose gel containing ethidium bromide. Electrophoresis was conducted at 60V for approximately 40 min, with a 1 kb marker in adjoining lanes.

Following electrophoresis, the gel was illuminated with long-wavelength ultraviolet light, and the band was excised from the gel. The gel slice was placed in a 0.5 ml tube, and the tube was heated at 68°C for 15 min. The temperature of the tube was then equilibrated at 45°C. (This is conveniently accomplished in a thermal cycler.) AgarACE™ (Promega) was then added to the tubes, according to the manufacturer's instructions, and incubation at 45°C was continued for 15 min. As a general rule, 2 μl of enzyme per 50 μl of gel slice is adequate. Following AgarACE™ digestion, the digestion mixture was extracted with phenol/chloroform according to the manufacturer's instructions, and nucleic acids were precipitated by addition of 0.1 volume of 3M NaOAc (pH 4.8), 20 μg glycogen, and 1.5 volumes of cold 95% ethanol. The precipitate was collected by centrifugation, dried and resuspended in 5 μl distilled water.

Gel-purified amplification products were cloned using the TOPO XL PCR Cloning Kit (Invitrogen) according to the manufacturer's instructions. After cloning, individual

colonies were selected at random for nucleotide sequence analysis of the inserts, using procedures for sequence determination that are well-known to those of skill in the art.

Example 2: Use of olfactory receptor polypeptides for screening

Components of a scent are identified by determining the interaction between one or more potential odorant molecules and one or more OR polypeptides. For example, if a known original scent involves binding to a particular set of ORs, any subsequent set of molecules which bind to that same set of ORs and stimulate or inhibit the response of the ORs to the same extent as the original scent is capable of re-creating that original scent. If each of the subsequent set of molecules interacts with one and only one OR, then the set of molecules is composed of receptor primary scent components. In similar fashion, scents which involve binding of multiple ORs can be recreated by identifying a molecule, or combination of molecules, which binds to that particular set of ORs.

Binding of molecules to ORs is determined by a number of methods that are well-known in the art including, but not limited to, in vitro and in silico methods as described herein. Binding of molecules to ORs can also be determined or approximated by using quantitative structure-activity relationships as described herein.

Example 3: Identification of agonists and antagonists of olfactory receptors

Interaction of an odorant with a particular OR embedded in the membrane of an olfactory neuron will activate a signaling cascade within the neuron, ultimately resulting in the perception of a particular smell. A molecule, produced for example by combinatorial chemistry, which activates a similar or identical signaling cascade, will induce the perception of the same smell. Such a molecule would be considered a OR agonist. An OR agonist, once identified, can be used as a probe to identify additional agonists, as well as antagonists, of that particular OR.

Assays for the activation and the end product(s) of signaling cascades are known in the art. For example, direct Ca^{++} imaging can be employed, using either dye -labeled Ca^{++} or dyes that are sensitive to Ca^{++} concentration. Such dyes, and techniques for their use, are available from, for example, Molecular Dynamics (Sunnyvale, CA) and Molecular Probes (Eugene, OR).

Because ORs are transmembrane proteins, identification of agonists and/or antagonists for a particular OR require that the OR is present either in a living cell or in a membrane preparation.

In one embodiment of a method for the determination of OR agonists or antagonists, a known OR agonist is labeled *in situ*, or is resynthesized with an attached label, and is bound to an OR. The effect of various test molecules on the binding of the labeled OR agonist is then determined. Labeling of an OR agonist is accomplished by any of a number of methods that are known to those of skill in the art including, but not limited to, various fluorescent labels (for example, chemical fluorochromes or green fluorescent protein). Binding of the OR agonist is measured by any of a number of competitive binding assays, as are known in the art. A test molecule that displaces the agonist from the OR (*i.e.*, reduces the binding of the agonist) is identified as a candidate agonist or antagonist of the particular OR. In a subsequent experiment, the candidate molecule is bound to the OR, and the effect on the signaling cascade induced by the original agonist is determined. A similar of higher level of activation is indicative of an agonist; while a reduced level of activation of the signaling cascade reflects the action of an antagonist.

In additional embodiments of the displacement assay, an unlabeled agonist is used, and its degree of binding is determined by mass spectrometry. *See*, for example, U.S. Patent No. 5,894,063; U.S. Patent No. 5,719,060; and Wei *et al.* (1999) *Nature* **399**:243-246.

In another embodiment, fluorescent microparticles ("beads"), which can be separated by flow cytometry, are used to identify OR agonists and antagonists. Such beads are available, for example, from Luminex (Austin, TX). Multiple different ORs are attached to the beads, wherein each distinct color of bead is associated with a particular OR. The collection of beads, containing different ORs, is exposed to a test molecule or a collection of test molecules, such as can be synthesized by combinatorial chemistry, and binding of the test molecule(s) is determined, for example, by use of a labeled ligand of the test molecule(s). The beads are sorted according to their color by flow cytometry. Correlation of test molecule binding with bead color allows the determination of test molecules capable of binding to the OR. Agonist or antagonist function of an OR binding molecule is determined by methods described *supra*.

Example 4: Summary of search parameters for homology searches

Step 1: (masking) rempolyatmask raw sequence on -NONE- [?] with remAT_moderate (15) . Continue to step 2.

Step 2: (masking) mask masked sequence from step 1 on RepBase [N] with mask_moderate (85) . Continue to step 3.

Step 3: (masking) mask masked sequence from step 2 on VecBase [N] with mask_moderate (85) . Continue to step 4.

Step 4: blastn masked sequence from step 3 on NR-Nuc [N] with blastn_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 5. Otherwise, stop.

Step 5: blastx masked sequence from step 3 on NR-Pro [P] with blastx_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 6. Otherwise, stop.

Step 6: blastn masked sequence from step 3 on GB_CurAwareness-Nuc [N] with blastn_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 7. Otherwise, stop.

Step 7: blastx masked sequence from step 3 on GB_CurAwareness-Pro [P] with blastx_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 8. Otherwise, stop.

Step 8: tblastx masked sequence from step 3 on NR-Nuc [N] with tblastx_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 9. Otherwise, stop.

Step 9: blastn masked sequence from step 3 on EST [N] with blastn_10_hits (V=10 B=10) . If the P/Z score is > 1.0E-50, or no hits are found go to step 10. Otherwise, stop.

Step 10: blastn masked sequence from step 3 on STS [N] with blastn_10_hits (V=10 B=10) . Stop.

Example 5: Summary of search results

Step	Program	Database	Score	Sequences By Best Hit's Score						No Hits	Run	Not Finished	Not Run
1	rempolyatmask	-NONE-[P]	P/Z/E	0	> 1.0 >=	0	>= 1.0 >	0		74	74	0	0
2	mask	RepBase[N]	P/Z/E	0	> 1.0 >=	0	>= 1.0 >	0		74	74	0	0
3	mask	VecBase[N]	P/Z/E	0	> 1.0 >=	0	>= 1.0 >	0		74	74	0	0
4	blastn	NR-Nuc[N]	P/Z/E	46	< 1.0E-20 <=	28				0	74	0	0
5	blastx	NR-Pro[P]	P/Z/E	16	< 1.0E-20 <=	34				0	50	0	24
6	blastn	GB_CurAwareness-Nuc[N]	P/Z/E	17	< 1.0E-20 <=	31				0	48	0	26
7	blastx	GB_CurAwareness-Pro[P]	P/Z/E	13	< 1.0E-20 <=	28				2	43	0	31
8	tblastx	NR-Nuc[N]	P/Z/E	14	< 1.0E-20 <=	29				0	43	0	31
9	blastn	EST[N]	P/Z/E	10	< 1.0E-20 <=	33				0	43	0	31
10	blastn	STS[N]	P/Z/E	5	< 1.0E-20 <=	33				0	38		

Example 6. Datamining and analysis from GenBank

Datamining. A datamining pipeline was built to detect all available OR-like sequences in the public databases and to update the results as new database versions are released. tblastn (Altschul et al., 1997) was used to compare amino acid query sequences to the non-redundant version of GenBank (partitions nt, htg and est_human, all updated to August 6th, 2000), with a non-stringent expectation value cutoff of 1e-4. The queries used included 96 curated OR sequences representing all known families (SEQ ID NO:2651 through SEQ ID NO:2747) and 249 additional HORDE entries (SEQ ID NO:2402 through SEQ ID NO:2650). In a second round 105 newly mined mouse genes (SEQ ID NO:2296 through SEQ ID NO:2401) and 344 newly mined human genes (SEQ ID NO:2009 through SEQ ID NO:2295) were used as additional queries (all datasets are available

electronically). All resulting database entries were catalogued by species and subdivided into four types: mRNA, EST, DNA and genomic, the latter including entries annotated with keyword HTGS_PHASE1-3, or with length at least 10 kb. Low-pass genomic sampling sequences were ignored (keyword HTGS_PHASE0). In addition, a set of 132 olfactory sequence tag (OST) sequences was used . All sequences used were split into contigs according to annotation or, where unavailable, according to runs of at least 50 Ns. All resulting contigs were analyzed for interspersed repeats using RepeatMasker (Smit and Green, 1997). Subcontigs were defined as segments between interspersed repeats, ignoring simple repeats and low-complexity regions.

Localization of genomic clones. The University of Santa Cruz (UCSC) Working Draft Sequence ("golden path", http://genome.ucsc.edu) presents a first tentative assembly of the finished and draft human genomic sequence based on the WUSTL clone map (http://genome.wustl.edu/gsc). The "golden path" data was used to assign a coordinate to each finished or unfinished genomic clone, in Mb from the p telomere. In parallel, the Unified DataBase (UDB) was used to assign similar Mb coordinates to the clones, based on their marker contents (Chalifa-Caspi et al., 1998). The two maps are largely colinear, and were integrated based on the coordinates of clones that could be localized in both. Clones for which no coordinate could be obtained by either method were assigned a chromosome according to UDB, by sequence similarity to another mapped clone, by annotation, or by e-PCR (Schuler, 1997).

Detection of OR sequences. Each subcontig was compared using FASTY (Pearson et al., 1997) to a curated set of OR protein sequences from several species, yielding a conceptual translation product. The possibility of a pseudogene being disrupted by the insertion of interspersed repeats was taken into account, with the two or more resulting parts being therefore located in different subcontigs. Such compatible candidate sequences were automatically joined into a combined reconstructed pseudogene. Whenever possible, all resulting sequences were trimmed or extended to use a suitable ATG codon for initiation and to end at a stop codon, but avoiding those stop codons that yield products shorter than 275 amino acids. The sequences were finally split into OR or non-OR by comparing them to previously recognized OR sequences and to a non-redundant database of non-OR GPCRs which we extracted from Swiss-Prot. To be automatically classified as an OR, a

new sequence has to be at least 40% identical over at least 100 amino acids to another OR. A more stringent cutoff (50%) was required for shorter sequences.

Definition of OR genes. A given gene could be represented in more than one overlapping genomic clone. Such redundancy was removed by considering two sequences as representing the same gene, if they are in the same chromosome, located in clones less than 300 kb apart and at least 99% identical at the nucleotide level. An exception to this rule is when two genes coappear in the same clone, in which case they were considered to be distinct genes. Sequences localized to a chromosome but without a coordinate were only compared to other sequences within that chromosome, and finally those sequences lacking a chromosomal assignment were compared to the rest, applying only the criterion of sequence similarity. For each resulting gene with more than one constituent sequence, a consensus nucleotide sequence was created after multiple alignment by ClustalW (Higgins et al., 1996) using the fast comparison parameter. This was followed by conceptual translation and end trimming to suitable start and stop codons, as above. Genes with length at least 275 amino acids without frame disruptions (frameshifts, in-frame stop codons or disrupting interspersed repeats) were considered to be full-length and apparently intact. For partial sequences without frame disruptions no statement could be made on their apparent functionality, except when the partial sequences were observed in the genome as such, in which case they were considered to be pseudogenes. Finally, each OR gene was assigned a family and subfamily by amino acid sequence similarity to previously classified OR genes.

The references cited in this example are: Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-402; Chalifa-Caspi, V., Prilusky, J. and Lancet, D. (1998) The Unified Database. Weizmann Institute of Science, Bioinformatics Unit and Genome Center (Rehovot, Israel). World Wide Web URL: bioinformatics.weizmann.ac.il/udb; Higgins, D. G., Thompson, J. D. and Gibson, T. J. (1996) Using CLUSTAL for multiple sequence alignments. Methods Enzymol 266: 383-402; Pearson, W. R., Wood, T., Zhang, Z. and Miller, W. (1997) Comparison of DNA sequences with protein sequences. Genomics 46: 24-36; Schuler, G. D. (1997) Sequence mapping by electronic PCR. Genome Res 7: 541 50; and Smit, A. F.

A. and Green, P. (1997) RepeatMasker at URL: repeatmasker.genome.washington.edu/cgi-bin/RM2_req.pl.

Tables 1 and 2 contain additional information regarding SEQ ID NO. 153 to SEQ ID NO. 1085. The explanation of the entries in Tables 1 and 2 is as follows:

Symbol: The Human Genome Organization gene symbol, as allotted by a procedure to be published soon. OR = Olfactory Receptor, numeral to the immediate right - family designation, capital letters - subfamily designation, rightmost numeral - individual gene within subfamily, n appearing when such number is not assigned yet; P = Pseudogene.

All ORs within a family share at least 40% protein sequence identity.

All ORs within a subfamily share at least 60% protein sequence identity.

HORDE: The H serial number within the Human Olfactory Receptor Data Exploratorium (URL bioinfo.weizmann.ac.il/HORDE). The numeral 38 represents the HORDE build (version), gxxx is the individual gene number.

Digi: Appearance of a DSnn serial number here means that the sequence has been PCR-amplified from human olfactory epithelial cDNA using degenerate primers at the transmembrane helix 2 and transmembrane helix 7. See separate page for explanations on the analysis of the DS entries.

OST: OSTnnn is the serial number of the sequence in the Olfactory Sequence Tag collection in the Lancet laboratory (URL bioinfo.weizmann.ac.il/HORDE). Appearance here means that the sequence has been PCR-amplified from human genomic DNA using degenerate primers at the transmembrane helix 2 and transmembrane helix 7. There are a total of 112 OST sequences.

Trivial name: One or more aliases given to the same gene by different laboratories. Many of the trivial names are of the form ORnn-xx, whereby nn is a chromosome number and xx is an arbitrary numerical identifier.

Tran: (transcribed) Plus appears if the entry was sequenced from cDNA, or was found in the Expressed Sequence Tags (EST) databases. Plus also appears if in the public databases the gene was annotated as mRNA.

Int.: (intact) "Yes" indicates that the gene may be intact, as there are no obvious sequence frame disruptions. "Put" (putative) indicates the same, except that the known sequence is short, hence there may be disruptions in the unsequenced segments. "Pol"

indicates a polymorphism between intact and pseudogenic alleles. When no word appears, this indicates a pseudogene.

E: (Extent) FL indicates that the Full Length sequence is known (typically 310 ± 30 amino acids).

D: The number of sequence disruptions in the known sequence of a pseudogene.

C: The human chromosomal location of the OR gene, assigned as described under Mb coord.

Mb coord: The location of the OR gene within a human chromosome, in magabase units, beginning at the p-telomere and ending at the q-telomere, computed based on integrating information from Unified Database (URL is bioinfo.weizmann.ac.il/udb) and the University of California Santa Cruz (URL is genome.ucsc.edu).

CDR: The 17 amino acids suggested to line the odorant ligand binding pocket, delineated by the extracellular 2/3 of transmembrane helices 3,4 and 5. The assignment is based on an algorithm at URL bioinformatics.weizman.ac.il/HORDE/humanGenes/CDR.html.

%: (% id) The percent protein identity between the human sequence in the current line and the known rodent (rat or mouse) OR sequence to which it bears the highest similarity.

S: (Species) Rat (R)or mouse (M).

Acc: The Genbank accession number of the clone that contains the rodent sequence.

Range: The positions x ... y of the first and last bases within the rodent which constitute the OR coding region. If x>y then the OR is on the reverse strand.

Table 1

SEQ ID #	Symbol	HORDE	Digi	OST	Trivial	Tran	Int.	E
153	OR10D3	H38g001			HSHTPCRX09			
154	OR7EnP	H38g002						FL
155	OR1D5	H38g003		OST901	OR17-31	+	pol	FL
156	OR10NnP	H38g00						FL

SEQ ID #	Symbol	HORDE	Digi	OST	Trivial	Tran	Int.	E
536	OR1ABnP	H38g385						
537	OR52MnP	H38g386						FL
538	OR1XnP	H38g387						FL
539	OR4FnP	H38g388						
540	OR52MnP	H38g389						FL
541	OR2Vn	H38g390					yes	FL
542	OR2V1P	H38g391		OST265				FL
543	OR2Zn	H38g392					yes	FL
544	OR52KnP	H38g393				+		
545	OR10Hn	H38g394					yes	FL
546	OR2Dn	H38g395					yes	FL
547	OR7EnP	H38g396						
548	OR11GnP	H38g397						FL
549	ORnP	H38g398						
550	OR11Gn	H38g399					yes	FL
551	OR11HnP	H38g400						FL
552	OR6Kn	H38g401					yes	FL
553	OR11Hn	H38g402					yes	FL
554	OR6KnP	H38g403						
555	OR11HnP	H38g404						FL

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ctgcgtttct ctgccacttt cccaccgatt ttggacacag ctgttgcact gatgtttgca 660
gttcttgctc cctttttcaa ccctatcatc tatagcttta gaaataagga catgaagatt 720
gcaattaaaa agcttttctg ccctcagaag atggttaatt tatctgta 768

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<212> DNA
<213> Unknown (H38g404 nucleotide)

<220>
<223> Synthetic construct

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<212> DNA
<213> Unknown (H38g405 nucleotide)

<220>
<223> Synthetic construct

<400> 556
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agttttatca gtgtcttctc cttcctggag atttggtaca ccaccgtgac tatccccaag 240
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CLAIMS

What is claimed is:

1. An isolated and purified polynucleotide sequence encoding an olfactory receptor and having the nucleotide sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO: 73 and SEQ ID NO:111 through SEQ ID NO:152, or a nucleotide sequence that is at least about 95% homologous to a nucleotide sequence of the group consisting of SEQ ID NO:1 through SEQ ID NO: 73 and SEQ ID NO:111 through SEQ ID NO:152 and encoding a polypeptide having olfactory receptor function.

2. An expression vector comprising a polynucleotide sequence of claim 1.

3. A host cell comprising the expression vector of claim 2.

4. An isolated and purified olfactory receptor polypeptide comprising the translated sequence of SEQ ID NO:1 through SEQ ID NO: 73 and SEQ ID NO:111 through SEQ ID NO:152, or a polypeptide sequence that is at least about 95% homologous to a polypeptide sequence of the group consisting of the translated sequence of SEQ ID NO:1 through SEQ ID NO: 73 and SEQ ID NO:111 through SEQ ID NO:152 and having olfactory receptor function.

5. A host cell expressing a polypeptide of claim 4 or a functional fragment thereof.

6. A phage expressing a polypeptide of claim 4 or a functional fragment thereof.

7. A preparation containing a polypeptide of claim 4, further comprising biological or synthetic molecules which maintain the functional structure of the polypeptide.

8. An isolated and purified polynucleotide sequence encoding an olfactory receptor and having the nucleotide sequence selected from the group consisting of SEQ ID NO: 153 through SEQ ID NO: 1084 or a nucleotide sequence having a sequence at least about 95% homologous to a nucleotide sequence of the group consisting of SEQ ID NO: 153 through SEQ ID NO: 1084 and encoding a polypeptide having olfactory receptor function.

9. An expression vector comprising a polynucleotide sequence of claim 8.

10. A host cell comprising the expression vector of claim 9.

11. An isolated and purified olfactory receptor polypeptide comprising the sequence of SEQ ID NO: 1085 through SEQ ID NO: 2008, or a polypeptide sequence that is at least about 95% homologous to a polypeptide sequence of the group consisting of SEQ ID NO: 1085 through SEQ ID NO: 2008 and having olfactory receptor function.

12. A host cell expressing a polypeptide of claim 11 or a functional fragment thereof.

13. A phage expressing a polypeptide of claim 11 or a functional fragment thereof.

14. A preparation containing a polypeptide of claim 11, further comprising biological or synthetic molecules which maintain the functional structure of the polypeptide.

15. A library of olfactory receptors suitable for determining the interaction pattern of a composition with the receptors, comprising the expression products of at least two polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084 wherein said polynucleotides encode functional olfactory receptors; or functional fragments of said expression products.

16. A library of olfactory receptors according to claim 15, wherein the library comprises the expression products of at least 50 polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084 wherein said polynucleotides encode functional olfactory receptors; or functional fragments of said expression products.

17. A library of olfactory receptors according to claim 15, wherein the library comprises the expression products of at least 100 polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084 wherein said polynucleotides encode functional olfactory receptors; or functional fragments of said expression products.

18. A library of olfactory receptors according to claim 15, wherein the library comprises the expression products of at least 200 polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084 wherein said polynucleotides encode functional olfactory receptors; or functional fragments of said expression products.

19. A library of olfactory receptors according to claim 15, wherein the library comprises the expression products of at least 500 polynucleotides of SEQ ID NO:1 through SEQ ID NO: 73, SEQ ID NO:111 through SEQ ID NO:152, and SEQ ID NO: 153 through SEQ ID NO: 1084 wherein said polynucleotides encode functional olfactory receptors; or functional fragments of said expression products.

20. A library of olfactory receptors suitable for determining the interaction pattern of a composition with the receptors, comprising at least two polypeptides of SEQ ID NO: 1085 through SEQ ID NO: 2008, wherein said polypeptides are functional olfactory receptors; or functional fragments of said polypeptides.

21. A library of olfactory receptors according to claim 20, wherein the library comprises at least 50 polypeptides of SEQ ID NO: 1085 through SEQ ID NO: 2008,

wherein said polypeptides are functional olfactory receptors; or functional fragments of said polypeptides.

22. A library of olfactory receptors according to claim 20, wherein the library comprises at least 100 polypeptides of SEQ ID NO: 1085 through SEQ ID NO: 2008, wherein said polypeptides are functional olfactory receptors; or functional fragments of said polypeptides.

23. A library of olfactory receptors according to claim 20, wherein the library comprises at least 200 polypeptides of SEQ ID NOS of SEQ ID NO: 1085 through SEQ ID NO: 2008, wherein said polypeptides are functional olfactory receptors; or functional fragments of said polypeptides.

24. A library of olfactory receptors according to claim 20, wherein the library comprises at least 500 polypeptides of SEQ ID NO: 1085 through SEQ ID NO: 2008, wherein said polypeptides are functional olfactory receptors; or functional fragments of said polypeptides.

25. A method for determining the binding pattern of a composition with olfactory receptors, comprising the steps of:

exposing the composition to a library according to claim 21; and

determining whether the composition binds to each olfactory receptor, thereby determining the overall binding patter of the composition.

26. The method of claim 25, wherein the composition consists essentially of one compound or chemical.

27. The method of claim 25, wherein the composition comprises at least two compounds or chemicals.

28. The method of claim 25, wherein the step of determining whether the composition binds to each olfactory receptor further comprises a determination of the

approximate binding constant with which the composition binds to each receptor or functional fragment thereof.

29. The method of claim 25, further comprising the step of determining whether a receptor or functional fragment thereof to which the composition binds is activated.

30. The method of claim 29, futher comprising the step of determining the absolute or relative amount by which the receptor or functional fragment thereof is activated.

31. A DNA array or a DNA chip comprising DNA segments derived from SEQ ID NO: 153 through SEQ ID NO: 1084.

32. A method of determining differences among individuals with respect to their olfactory faculties, comprising the steps of comparing the olfactory DNA of the individual against the array or chip of claim 31.

33. A method to determine single nucleotide polymorphisms in olfactory receptors, comprising the steps of uniquely amplifying olfactory receptor sequences from DNA obtained from one or more individuals, based on primers designed according to the first 25 bases and the last 25 bases of any combination of, or each of, SEQ ID NO: 153 through SEQ ID NO: 1084, and determining the similarities and differences between said amplified DNA and the corresponding receptor from SEQ ID NO: 153 through SEQ ID NO: 1084.